

B. Braun Medical AG
Centre of Excellence Infection Control
Seesatz 17
CH-6204 Sempach

Bremen, 02/08/2017

Expert opinion

Adenovirus efficacy of Promanum pure in a quantitative suspension test at 20°C according to EN 14476 under clean conditions

This updated expert opinion is based on the test report B11ML1291AA dated 17/12/2011.

The virus-inactivating properties of the hand disinfectant Promanum pure of B. Braun Medical AG against adenovirus type 5 were investigated by a quantitative suspension test according to prEN 14476:2011 under clean conditions.

According to EN 14476 a disinfectant or a disinfectant solution at a particular concentration is considered as having virus-inactivating properties if within the recommended exposure period the titer is reduced by $\geq 4 \log_{10}$ (inactivation $\geq 99.99\%$).

Promanum pure was examined undiluted at 20°C. 30 and 60 seconds were chosen as exposure times. The hand disinfectant Promanum pure of B. Braun Medical AG demonstrated effectiveness against adenovirus type 5 undiluted after a contact time of 30 seconds at 20°C under clean conditions. Therefore, Promanum pure can be declared as virucidal against adenovirus as follows:

undiluted 30 seconds

The test report still fulfills the requirements for a measurement of virucidal activity based on EN 14476:2013+A1:2015, which represents the ~~currently valid~~ test standard.


Dr. Jochen Steinmann

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Bremen, 2. August 2017

Gutachten

Wirksamkeit von Promanum pure gegenüber dem Adenovirus Typ 5 im quantitativen Suspensionsversuch gemäß EN 14476 unter geringer organischer Belastung

Dieses aktualisierte Gutachten basiert auf dem Prüfbericht B11ML1291AA vom 17.12.2011.

Das Händedesinfektionsmittel Promanum pure der B. Braun Medical AG wurde gemäß Auftrag auf seine virusinaktivierenden Eigenschaften gegenüber dem Adenovirus Typ 5 nach der prEN 14476:2011 unter geringer Belastung untersucht.

In der EN 14476 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 Händedesinfektionsmittel Promanum pure wurde unverdünnt bei 20°C untersucht. Die Einwirkzeiten betrugen 30 und 60 Sekunden. Nach 30 Sekunden war eine Reduktion des Virustiters um $\geq 4 \log_{10}$ -Stufen unter geringer Belastung in allen Ansätzen nachweisbar. Somit ergibt sich eine Adenovirus-Wirksamkeit wie folgt:

unverdünnt 30 Sekunden

Der genannte Prüfbericht erfüllt ebenfalls die Voraussetzungen für die Auslobung einer ausreichenden Wirksamkeit gemäß EN 14476:2013+A1:2015, die den aktuell gültigen Teststandard darstellt.

Dr. Jochen Steinmann





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ZLG-P-429.08.10

17.12.2011

Test report B11ML1291AA

Evaluation of the effectiveness of **Promanum pure**

Test virus: adenovirus type 5

Method: prEN 14476:2011

TEST REPORT

Sponsor:

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1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the hand disinfectant Promanum pure against adenovirus type 5 using a quantitative suspension assay according to prEN 14476:2011 (1) under clean conditions.

2. Test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of the sample

Manufacturer	B. Braun Medical AG
Name of product	Promanum pure
Batch number	0074M08
Application	hand disinfectant
Date of production	18.02.2010
Expiry date	01.2015
Active compound (s) (100 g)	73.4 g ethanol 10.0 g propan-2-ol
Appearance and odour	clear, colourless liquid; product specific
pH-value (s) in WSH	undiluted: 5.72 (20°C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	02.09.2011

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).



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)
- Polystyrol 96-well microtiter plates (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Cell culture flasks (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	undiluted (80.0 %) and as 50.0 % and 10.0 % (non-active range) solutions
Contact time(s)	30 and 60 seconds
Interfering substance(s)	clean conditions: 0.3 g/l bovine serum albumin (BSA)
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	02.09.2011 – 06.12.2011
End of testing	06.12.2011



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 evaluated undiluted. Due to the addition of test virus suspension and interfering substance an 80.0 % solution resulted. The product was additionally tested as 50.0 % and 10.0 % (non-active range) solutions.

The 50.0 % and 10.0 % solution was prepared with water of standardised hardness immediately before the inactivation tests.

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 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 prEN 14476:2011, 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.

6.5 Inactivation assay

Investigations for determination of virucidal activity followed EN 6.6. Promanum pure was examined undiluted (80.0 %) and as 50.0 % and 10.0 % solutions at 20°C. 30 and 60 seconds were chosen as exposure times.

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).

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

Inactivation tests were carried out in sealed test tubes in a water bath at $20^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$. Aliquots were retained after appropriate exposure times, and residual infectivity was determined.

6.6 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.

6.7 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 re-suspended 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.

6.8 Control of efficacy for suppression of disinfectant activity

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

6.9 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 undiluted test product showed cytotoxicity in the 1:10 dilutions thus allowing the detection of a four \log_{10} reduction of the 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: 8.00 ± 0.44 (PBS) versus 7.88 ± 0.37 (disinfectant) \log_{10} TCID₅₀/ml.
- d) The control of efficacy for suppression of disinfectant activity demonstrated no decrease of virus titre.

Since all criteria according to EN 8.3 were fulfilled, examination with adenovirus type 5 according to prEN 14476:2011 was valid.

8. Results

The hand disinfectant Promanum pure was examined undiluted (80.0 %) and as 50.0 % and 10.0 % solutions at 20°C.



Results of examinations are shown in tables 1 to 8. Tables 1 to 7 demonstrate the raw data, whereas table 8 gives a summary of results.

Promanum pure (undiluted) was able to inactivate adenovirus type 5 after 30 seconds under clean conditions in a quantitative suspension test. The reduction factors achieved $\geq 5.38 \pm 0.25$ and $\geq 5.13 \pm 0.29$ after this time point (Tables 1 and 2). This corresponded to an inactivation of $\geq 99.999\%$ (mean value $\geq 5.26 \pm 0.19$).

The 50.0 % solution was additionally able to inactivate adenovirus type 5 after 30 seconds under clean conditions in a quantitative suspension test. The reduction factor was $\geq 4.00 \pm 0.48$ after this time point (Table 2).

The 10.0 % solution was not able to inactivate adenovirus type 5 after 60 seconds of exposure time (Tables 3).

9. Summary

The hand disinfectant Promanum pure of B. Braun Medical AG demonstrated effectiveness against adenovirus type 5 undiluted after a contact time of 30 seconds under clean conditions. Therefore, Promanum pure can be declared as active against adenovirus type 5 under clean conditions as follows:

undiluted 30 seconds

Bremen, 17.12.2011


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 170+3). 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. Records 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.



12. Literature

1. prEN 14476:2011: 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



Appendix

Table 1:	Raw data of Promanum pure (80.0 %) tested against adenovirus type 5 under clean conditions (1 st assay)
Table 2:	Raw data of Promanum pure (80.0 %) tested against adenovirus type 5 under clean conditions (2 nd assay)
Table 3:	Raw data of Promanum pure (50.0 %) tested against adenovirus type 5 under clean conditions
Table 4:	Raw data of Promanum pure (10.0 %) tested against adenovirus type 5 under clean conditions
Table 5:	Raw data of formaldehyde solution (0.7 %) tested against adenovirus type 5
Table 6:	Control of efficacy for suppression of disinfectant activity (80.0 %)
Table 7:	Raw data (adenovirus type 5) for cell sensitivity to virus (80.0 %)
Table 8:	Results with Promanum pure and adenovirus type 5 (summary)



Table 1: Raw data of Promanum pure (80.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2702)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)							
				1	2	3	4	5	6	7	8
product	80.0%	clean conditions	0.5	tttt tttt	0000 2000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d. 0000
			1	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d. 0000
			2	n.d.	n.d.	n.d.	n.d.	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.
product cytotoxicity	80.0%	clean conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d. 0000	n.d. 0000	n.d. 0000
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7% (m/V)	PBS	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.
			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.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4334 2334	3002 0013	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4333 3343	3332 0000	0000 0000
virus control	n.a.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4334 2334	3002 0013	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4333 3343	3332 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 Promanum pure (80.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2761)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
product	80.0%	clean conditions	0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d. n.d.
			1	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d. n.d.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	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.
product cytotoxicity	80.0%	clean conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d. 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.
			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.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3332	3300 0020	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3243 0334	0030 0040	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 of Promanum pure (50.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2761)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
product	50.0%	clean conditions	0.5	n.d.	4444 4444	0000 0030	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			1	n.d.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	
			2	n.d.	n.d.	n.d.	n.d.	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.	
product cytotoxicity	50.0%	clean conditions	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.	
			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.	
			60	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.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3332	3300 0020	0000 0000	
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3243 0334	0030 0040	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 of Promanum pure (10.0 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2761)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
product	10.0%	clean conditions	0.5	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4443 4344	2300 0000	0002 2000	n.d. n.d.
			1	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3344 4224	0000 3200	0000 0000	n.d. n.d.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	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.
product cytotoxicity	10.0%	clean conditions	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.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3334 3332	3300 0020	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3243 0334	0030 0040	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 formaldehyde solution (0.7 %) tested against adenovirus type 5 (quantal test; 8 wells) at 20°C (2761)

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.
			10	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	tttt tttt	4444 4444	4444 4444	4444 4444	3330 1344	1000 0030	0000 0000	n.d.	
			15	tttt tttt	tttt tttt	4444 4444	3434 4444	0000 0200	0000 0000	0000 0000	n.d.		
			30	tttt tttt	tttt tttt	4333 2233	0030 0002	0000 0021	0000 0000	0000 0000	n.d.		
			60	tttt tttt	tttt tttt	0010 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	4444 4434	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 6: Control of efficacy for suppression of disinfectant activity (80.0 %) (2761)

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	clean	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	4444 3444	3030 0004	2000 0000	0000 0000
product	dirty	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 7: Raw data (adenovirus) for cell sensitivity to virus (80.0 %) (2761)

Product	Interfering substance	Dilution	Dilutions (log ₁₀)								
			1	2	3	4	5	6	7	8	9
PBS	clean conditions	without	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4333 1443	0002 3002	0000 0004	0000 0000
test product	PBS	1:10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	clean conditions	1:10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:100	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3444 4444	0030 3003	0000 0000	0000 0000
test product	dirty conditions	1:10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:100	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 8: Results with Promanum pure and adenovirus type 5 (summary)

Product	Con- centration	Interfering substance	Level of cytotoxicity	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	
Test product	80.0%	clean conditions	2.50	n.d.	≤2.63 ±0.25	≤2.50 ±0.00	n.d.	n.d.	n.d.	n.d.	0.5 (≥5.38 ±0.25)
Test product	80.0%	clean conditions	2.50	n.d.	≤2.50 ±0.00	≤2.50 ±0.00	n.d.	n.d.	n.d.	n.d.	0.5 (≥ 5.13 ±0.29)
Test product	50.0%	clean conditions	1.50	n.d.	3.63 ±0.25	≤2.50 ±0.00	n.d.	n.d.	n.d.	n.d.	0.5 (≥ 4.00 ±0.48)
Test product	10.0%	clean conditions	1.50	n.d.	8.00 ±0.46	7.75 ±0.33	n.d.	n.d.	n.d.	n.d.	> 1.0
Form- aldehyde	0.7% (m/V)	PBS	3.50	n.d.	n.d.	n.d.	n.d.	7.63 ±0.41	6.38 ±0.41	5.00 ±0.46	≤3.63 ±0.25 60

n.a. = not applicable

n.d. = not done



Product	Con- centration	Interfering substance	Level of cytotoxicity	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	
Virus control	n.a.	clean conditions	n.a.	8.00 ±0.38 7.88 ±0.37	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.00 ±0.38 7.63 ±0.41	n.a.
Virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.63 ±0.25	n.a.
Suppression control	80.0%	clean conditions	2.50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.00	n.d.	n.a.
Cell sens. PBS	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.00 ±0.44	n.a.
Cell sens. disinfectant	80.0% → 1:100	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.88 ±0.37	n.a.

n.a. = not applicable

n.d. = not done