

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

Bremen, 1. November 2016

## Gutachten

Wirksamkeit von Promanum pure gegenüber dem Poliovirus Typ 1 im quantitativen Suspensionsversuch nach der EN 14476:2013+A1:2015 unter geringer Belastung

Dieses Gutachten basiert auf dem Prüfbericht L16/0758bPo.1 vom 01.11.2016.

Das Händedesinfektionsmittel Promanum pure der B. Braun Medical AG wurde gemäß Auftrag auf seine virusinaktivierenden Eigenschaften gegenüber dem Poliovirus Typ 1 im quantitativen Suspensionsversuch gemäß EN 14476 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, 60, 120 und 180 Sekunden. Zusammenfassend ergibt sich eine Wirksamkeit gegenüber dem Poliovirus Typ 1 wie folgt:

**unverdünnt    60 Sekunden    geringe Belastung**

**Dr. Jochen Steinmann**

**Promanum pure – EN 14476:2013+A1:2015**

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

Bremen, 01/11/2016

## Expert opinion

Activity of Promanum pure against poliovirus type 1 in a quantitative suspension test according to the EN 14476:2013+A1:2015 under clean conditions

This expert opinion is based on the test report L16/0758bPo.1 dating 01/11/2016.

The virus-inactivating properties of the hand disinfectant Promanum pure of B. Braun Medical AG against poliovirus type 1 were investigated by a quantitative suspension test according to EN 14476 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 titre is reduced by  $\geq 4 \log_{10}$  (inactivation  $\geq 99.99\%$ ).

Promanum pure was examined undiluted at 20 °C. 30, 60, 120 and 180 seconds were chosen as exposure times. In summary, a virucidal activity against poliovirus type 1 was measured as follows:

**undiluted      60 seconds      clean conditions**

**Dr. Jochen Steinmann**

**Promanum pure – EN 14476:2013+A1:2015**



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



01/11/2016

## Test report L16/0758bPo.1

Evaluation of the effectiveness of  
**Promanum pure**

Test virus: poliovirus type 1 strain LSc-2ab

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:

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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	B. Braun Medical AG
Name of product	Promanum pure
Product diluent recommended by the manufacturer	-
Batch number / lab intern number	0074M08 / ML1291/11 14074M18 / L16/0758.B
Application	hand disinfection
Production date	18/02/2010 -
Expiry date	01/2015 01/2019
Active compound (s) (100 g)	73.4 g ethanol 10.0 g propan-2-ol
Appearance, odour	clear, colorless liquid product specific
pH-values	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 14/10/2016

## 3. Materials

### 3.1 Culture medium and reagents

- Dulbecco's Modified Eagle's Medium (DMEM, Biozym Scientific GmbH, catalogue no. 880021)
- 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 poliovirus type 1 strain LSc-2ab (Chiron-Behring) was obtained from PD Dr. Olaf Thraenhardt, Eurovir, DE - 14943 Luckenwalde.

*BGM cells* (buffalo green monkey = permanent monkey kidney cell line; supplied by Prof. Dr. Lindl, Institut für angewandte Zellkultur, DE - 81669 München, Germany) were cultivated in a 175 cm<sup>2</sup> flask with Dulbecco's Modified Eagle's Medium (DMEM) and 10 % fetal calf serum (FCS).

The cells (passage 13) 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<sub>2</sub> 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)
- Polystyrol 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 (97.0 % and 80.0 %) and 10.0 % solution (non-active range)
Appearance of product dilutions	no precipitation
Contact times	30, 60, 120 and 180 seconds
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 (97.0 % solution)	minor clouding, no precipitation
Virus strain	poliovirus type 1 strain LSc-2ab (Chiron-Behring)
Date of testing	02/09/2011 – 17/12/2011 14/10/2016 – 01/11/2016
End of testing	01/11/2016

#### 5. Methods

##### 5.1 Preparation of test virus suspension

For preparation of test virus suspension according to EN 5.4.1 *BGM 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. Additionally, the test product was examined as 97.0 % solution (0.1 part test virus suspension + 0.2 part interfering substance (5-fold) + 9.7 parts disinfectant).

Furthermore, the product was evaluated as 10.0 % solution (demonstrating of non-active range). This solution was 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 to 0.1 ml of freshly trypsinised *BGM cells* ( $10\text{--}15 \times 10^3$  cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO<sub>2</sub>-atmosphere. The cytopathic effect was read by using an inverted microscope after seven days. Calculation of the infective dose TCID<sub>50</sub>/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

$X_0$  = log<sub>10</sub> of the lowest dilution with 100 % positive reaction

$r$  = number of pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

$n$  = number of determinations for each dilution step.

### 5.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to the EN 14476, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by four log<sub>10</sub> steps within the recommended exposure period. This corresponds to an inactivation of  $\geq 99.99$  %.

### 5.5 Inactivation assay

Determination of virucidal activity has been carried out in accordance to EN 5.5. The test product was examined undiluted (97.0 % and 80.0 %) and 10.0 % (demonstration of non-active range) solution in water at 20 °C according to EN 14476. 30, 60, 120 and 180 seconds were chosen as contact times.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10<sup>-8</sup>.

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

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WSH or Aqua bidest. (RTU products). For the 97.0 % assays 0.1 parts by volume of test virus suspension were mixed with 0.2 parts interfering substance and 9.7 parts by volume of WSH or Aqua bidest. (RTU products).

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

Inactivation tests were carried out in sealed test tubes in a water bath at  $20\text{ °C} \pm 1.0\text{ °C}$ . 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 0.3 parts by volume hard water were mixed with 9.7 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  $37\text{ °C}$  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  $\geq 5.00 \pm 0.25$ ).
- b) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test (see 6.6.7) was  $1.75 \pm 0.58$  (between 0.5 - 2.5) after 30 min and  $2.13 \pm 0.57$  (between 2.0 - 4.5) after 60 min for poliovirus type 1.
- c) The test product (97.0 % and 80.0 %) showed cytotoxicity in the 1:10 dilutions thus allowing the detection of a  $4 \log_{10}$  reduction of virus titre.
- d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *BGM cells* showed no significant difference ( $< 1 \log_{10}$ ; EN 5.7) of virus titre:  $8.13 \pm 0.38$  (PBS) versus  $8.13 \pm 0.38$  (1:100 dilutions of disinfectant, 97.0 %)  $\log_{10}$  TCID<sub>50</sub>/ml.
- e) The control of efficacy for suppression of disinfectant's activity (97.0 % solution) showed no decrease ( $\leq 0.5 \log_{10}$ ; EN 5.5.5.1) in virus titre ( $7.25 \pm 0.33$  versus  $7.50 \pm 0.33 \log_{10}$  TCID<sub>50</sub>/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 poliovirus type 1 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 (97.0 % assay) was able to inactivate poliovirus type 1 after 60 seconds under clean conditions in this quantitative suspension test (tables 1 and 2). The reduction factors were  $\geq 4.13 \pm 0.61$  and  $\geq 4.37 \pm 0.61$  (mean  $\geq 4.25 \pm 0.43$ ). This value corresponded to an inactivation of  $\geq 99.99$  %

The undiluted test product in an 80.0 % assay was not able to inactivate poliovirus type 1 within 180 seconds under clean conditions in this quantitative suspension test (table 5).

Tested as 10.0 % solution, the test product was not active within 180 seconds of exposure time (table 6).

## 8. Conclusion

The hand disinfectant Promanum pure tested undiluted demonstrated effectiveness against poliovirus type 1 after an exposure time of 60 seconds under clean conditions.

Therefore, the hand disinfectant Promanum pure can be declared as active against poliovirus type 1 as follows:

**undiluted    60 seconds    clean conditions**

**Bremen, 01/11/2016**

**- 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)
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## Appendix:

### Legend to the Tables

Table 1:	Raw data for Promanum pure (97.0 %) tested against poliovirus type 1 (1 <sup>st</sup> assay)
Table 2:	Raw data for Promanum pure (97.0 %) tested against poliovirus type 1 (2 <sup>nd</sup> assay)
Table 3:	Raw data for Promanum pure (97.0 %) tested against poliovirus type 1 (3 <sup>rd</sup> assay)
Table 4:	Raw data for Promanum pure (97.0 %) tested against poliovirus type 1 (4 <sup>th</sup> assay)
Table 5:	Raw data for Promanum pure (80.0 %) tested against poliovirus type 1
Table 6:	Raw data for Promanum pure (10.0 %) tested against poliovirus type 1
Table 7:	Raw data for formaldehyde solution (0.7 %) tested against poliovirus type 1
Table 8:	Raw data for control of efficacy for suppression of disinfectant's activity (97.0 %)
Table 9:	Raw data (poliovirus type 1) for cell sensitivity (97.0 %)
Table 10 (a+b):	Summary of results with Promanum pure and poliovirus type 1

### Legend to the Figures

Figure 1:	Virus-inactivating properties of Promanum pure (97.0 %)
Figure 2:	Virus-inactivating properties of formaldehyde (0.7 %)

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**Table 1: Raw data for Promanum pure (97.0 %) (ML1291/11) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (#2765) (1<sup>st</sup> assay)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
test product	97.0 %	clean conditions	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1	tttt tttt	0440 4404	0044 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			2	tttt tttt	4000 0040	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	97.0 %	clean 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.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0040 0004	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4440	4000 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)

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**Table 2: Raw data for Promanum pure (97.0 %) (ML1291/11) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (#2765) (2<sup>nd</sup> assay)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
test product	97.0 %	clean conditions	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1	tttt tttt	0000 0444	0004 0400	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			2	tttt tttt	0004 4040	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	97.0 %	clean 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.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0040 0004	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4440	4000 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)

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**Table 3: Raw data for Promanum pure (97.0 %) (L16/0758b) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (#4663) (3<sup>rd</sup> assay)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
test product	97.0 %	clean conditions	0.5	tttt tttt	4444 4444	4444 4444	0404 0400	0000 0000	0000 0000	0000 0000	n.d. n.d.	n.d. n.d.
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	97.0 %	clean 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.	clean conditions	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	4400 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)

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**Table 4: Raw data for Promanum pure (97.0 %) (L16/0758b) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (#4663) (4<sup>th</sup> assay)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
test product	97.0 %	clean conditions	0.5	tttt tttt	4444 4444	4444 4444	4440 0444	4000 0000	0000 0000	0000 0000	n.d. 0000	n.d. 0000
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	97.0 %	clean 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.	clean conditions	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	0040 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)

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**Table 5: Raw data for Promanum pure (80.0 %) (ML1291/11) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (#2765)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	clean conditions	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	4444 0344	0004 0400	0000 0000	n.d.
			2	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4444	4000 0400	0004 0000	0000 0000	n.d.
			3	tttt tttt	4444 4444	4444 4444	4444 4444	4000 4444	0000 4000	4000 0000	0000 0000	n.d.
test 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.
virus control	n.a.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0440 0404	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4404 4044	4000 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)

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**Table 6: Raw data for Promanum pure (10.0 %) (ML1291/11) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (#2765)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
test product	10.0 %	clean conditions	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0044 4044	0000 0004	n.d.
			2	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4400	0400 0000	n.d.
			3	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0440 4044	0000 0000	n.d.
test product cytotoxicity	10.0 %	clean conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0440 0404	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4404 4044	4000 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)

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**Table 7: Raw data for formaldehyde solution (0.7 %) tested against poliovirus type 1 at 20 °C (quantal test; 8 wells) (#4663)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7 % (m/V)	PBS	5	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4004	0400 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0004 0004	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	4444 4444	4444 4444	0400 4440	0000 0400	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	4444 4444	4444 4044	0040 0044	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 4444	0404 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)

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**Table 8: Raw data for control of efficacy for suppression of disinfectant's activity (97.0 %) (#2765) (ML1291/11)**

Product	Interfering substance	dilutions (log <sub>10</sub> )								
		1	2	3	4	5	6	7	8	9
test product	clean conditions	tttt	4444	4444	4444	4444	4404	0000	0000	n.d.
		tttt	4444	4444	4444	4444	0444	0000	0000	
corresponding virus control	clean conditions	4444	4444	4444	4444	4444	4444	4000	0000	0000
		4444	4444	4444	4444	4444	4440	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)

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**Table 9: Raw data (poliovirus type 1) for cell sensitivity (97.0 %) (#2765) (ML1291/11)**

Product	Dilution	Dilutions (log <sub>10</sub> )								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 4030	0004 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	4444 4444	4404 0040	0000 4000	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)

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**Table 10a: Summary of results with Promanum pure and poliovirus type 1**

Product	Con- centration	Interfering substance	Level of cytotoxicity	log <sub>10</sub> TCID <sub>50</sub> /ml after ... min					> 4 log <sub>10</sub> reduction after ...min
				0.5	1	2	3	30	
test product (1)	97.0 %	clean conditions	2.50	n.d.	≤ 3.38±0.49	≤ 2.75±0.33	n.d.	n.d.	1 (RF ≥ 4.13±0.61)
test product (2)	97.0 %	clean conditions	2.50	n.d.	≤ 3.13±0.49	≤ 2.88±0.37	n.d.	n.d.	1 (RF ≥ 4.38±0.61)
test product (3)	97.0 %	clean conditions	2.50	4.88±0.37	n.d.	n.d.	n.d.	n.d.	> 0.5 (RF = 1.88±0.49)
test product (4)	97.0 %	clean conditions	2.50	5.38±0.41	n.d.	n.d.	n.d.	n.d.	> 0.5 (RF = 1.25±0.48)
test product	80.0 %	clean conditions	2.50	n.d.	7.63±0.41	6.88±0.41	6.38±0.51	n.d.	> 3
test product	10.0 %	clean conditions	1.50	n.d.	8.25±0.44	8.38±0.41	8.13±0.37	n.d.	> 3

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

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**Table 10b: Summary of results with Promanum pure and poliovirus type 1**

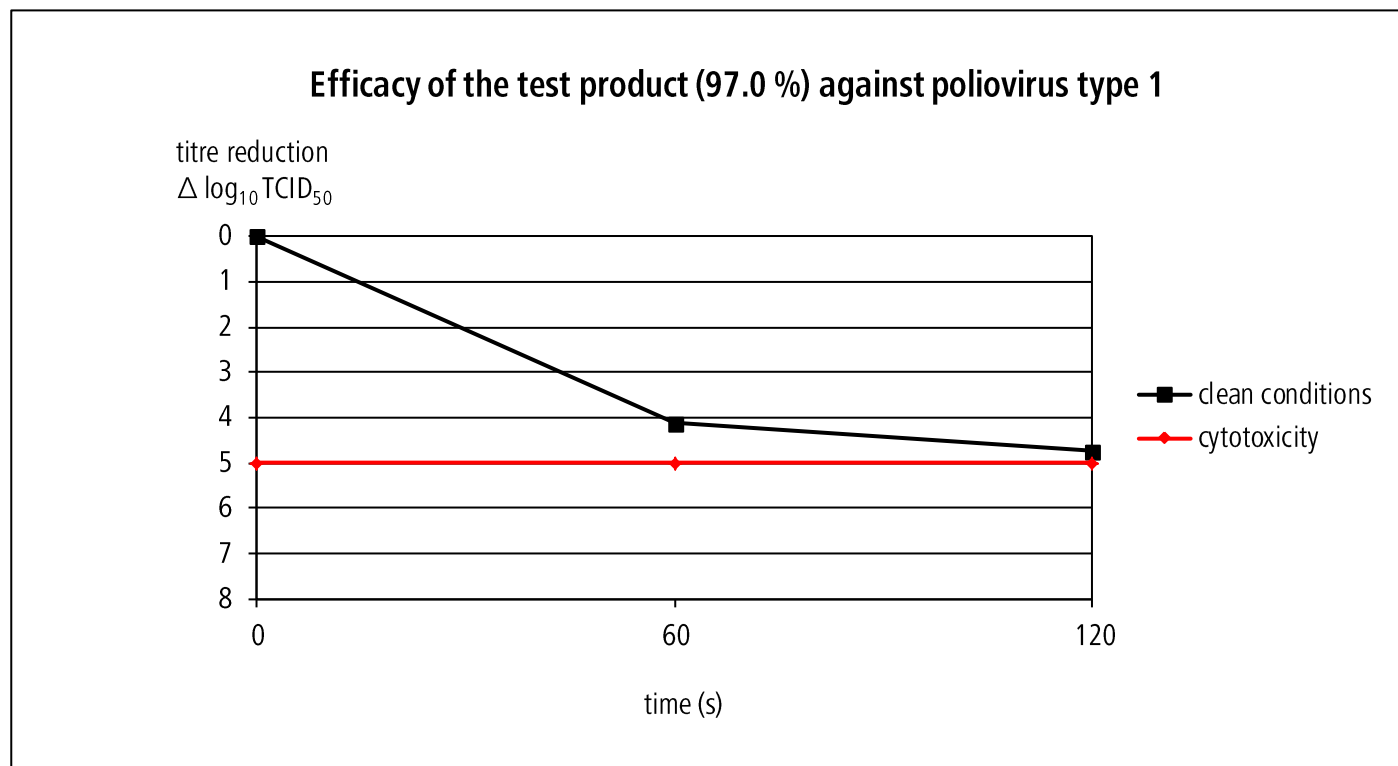
Product	Con- centration	Interfering substance	Level of cytotoxicity	log <sub>10</sub> TCID <sub>50</sub> /ml after ....min					> 4 log <sub>10</sub> reduction after ... min
				0	5	15	30	60	
formaldehyde	0.7% (w/v)	PBS	3.50	n.d.	7.38±0.41	6.75±0.33	6.13±0.45	5.75±0.44	> 60
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.88±0.37	n.a.
virus control (1)	n.a. (97.0 % assay)	clean conditions	n.a.	6.75±0.33	n.d.	n.d.	n.d.	7.50±0.35	
virus control (2)	n.a. (97.0 % assay)	clean conditions	n.a.	6.75±0.33	n.d.	n.d.	n.d.	7.50±0.35	n.a.
virus control (3)	n.a. (97.0 % assay)	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.33	n.a.
virus control (4)	n.a. (97.0 % assay)	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.25	n.a.
virus control	n.a. (80.0 % assay)	clean conditions	n.a.	8.00±0.38	n.d.	n.d.	n.d.	8.38±0.41	n.a.
suppression control	97.0%	clean conditions	2.50	n.d.	n.d.	n.d.	7.25±0.38	n.d.	n.a.
sens.control PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	8.13±0.38	n.a.
sens. control test product	97.0% → 1:100	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	8.13±0.38	n.a.

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

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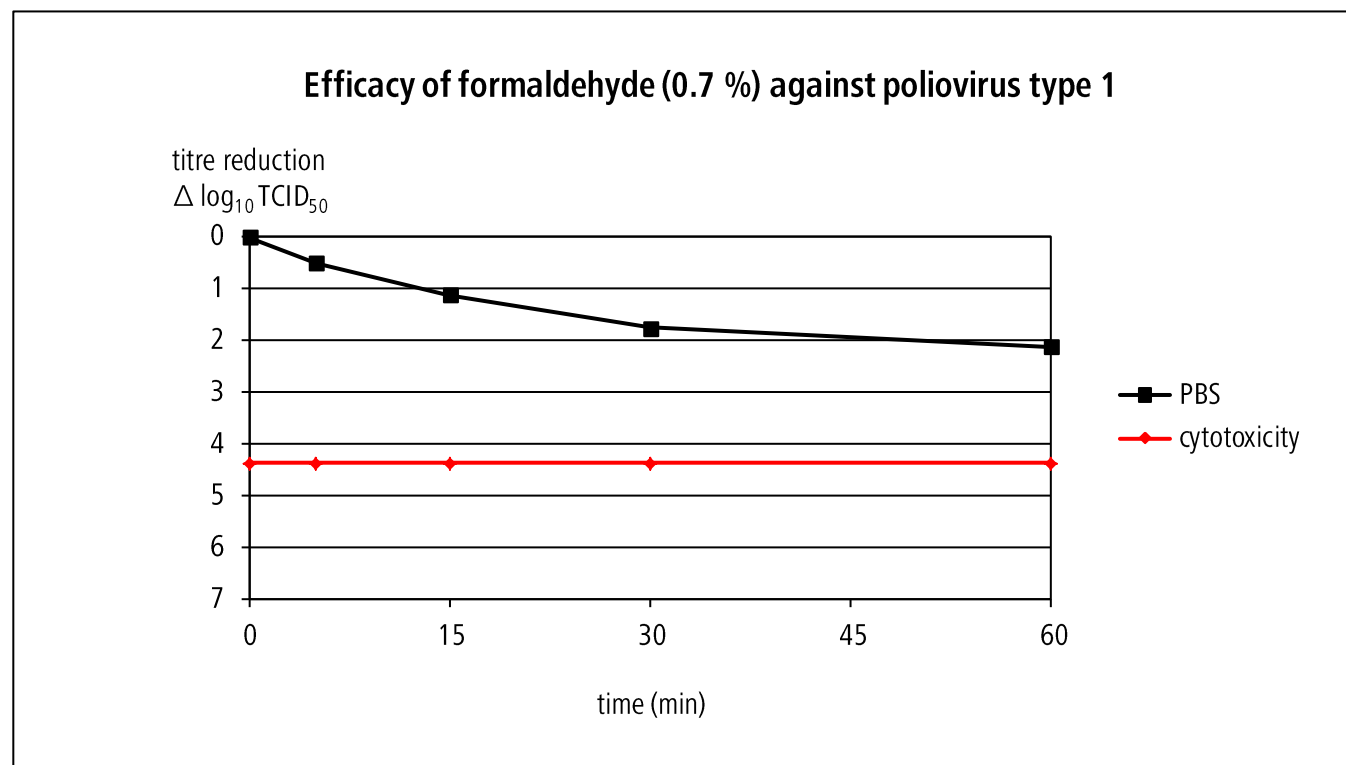


**Figure 1: Virus-inactivating properties of Promanum pure (97.0 %)**



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



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