DR. BRILL + DR. STEINMANN
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE

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TEST REPORT

10.06.2015

Test report C15L0096aM

Evaluation of the effectiveness of

Chemides Pulver

Test virus: murine norovirus (as surrogate of human norovirus)

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

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Date: 2025.11.12 14:00:20 EET
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1. Identification of test laboratory

Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Norderoog 2, D-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

- Dulbecco's Modified Eagle's Medium (DMEM, Biozym Scientific GmbH, catalogue no. 880006)
- Fetal calf serum (Thermo Fisher, article no. CH30160.02)
- 1.4 % formaldehyde solution (dilution of Roti®-Histofix 4 %, Carl Roth GmbH)
- 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).

3.2 Virus and cells

Murine norovirus (MNV) was obtained from PD. Dr. E. Schreier, Head of FG15 Molecular Epidemiology of Viral Pathogens at the Robert Koch-Institute (RKI) in Berlin. Prior to inactivation, MNV was passaged three times in *RAW 264.7 cells* (a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice, ATCC TIB-71). RAW 264.7 cells were cultured with Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose and fetal calf serum with low endotoxin.

Furthermore, cells (passage 17) 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 % and 0.1 % (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 (1.0 % solution)	noflocculation, noprecipitation
Virus strain	murine norovirus (Berlin 06 / 06 / DE Isolate S99)
Date oftesting	23.02.2015– 10.06.2015
End of testing	10.06.2015

5. Methods

5.1 Preparation of test virus suspension

To prepare the test virus suspension, *RAW 264.7 cells* which have been cultured with Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose and 10 % fetal calf serum with low endotoxin were inoculated with MNV (stock virus solution) in a 175 cm^2 cell culture flask. Once a cytopathic effect had been induced (approx. 1-3 days), freezing and thawing was carried out two times. The cell debris was removed by low speed centrifugation (400 g_N and 15 min) and the supernatant was recovered as test viral suspension, aliquoted and 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 % and 0.1 %) 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 to 0.1 ml of freshly trypsinised *RAW 264.7 cells* (10-15 x 10^3 cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after five 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 0.75 %, 1.0 %, 0.5 % and 0.1 % (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}.

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 ≥ 5.13± 0.29).

b) The test product (1.75 % and 1.0 %) showed not cytotoxicity in the 1:10 dilutions thus allowing the detection of a 4 log_{10} reduction of virus titre.

c) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *RAW 264.7 cells* showed no significant difference (< 1 log_{10}; EN 5.7) of virus titre: 7.50± 0.46 (PBS) versus 7.50± 0.46 (1:100 dilutions of disinfectant as 1.75 % solution) $log_{10}TCID_{50}$/ml. The 1:10 dilutions showed cytotoxicity. Therefore, this dilution cannot be considered for the calculation of the reduction factor.

d) The control of efficacy for suppression of disinfectant's activity (1.75 % solution) showed no decrease (<0.5 log_{10}; EN 5.5.5.1) in virus titre (7.13 ± 0.45 versus 7.63 ± 0.41 $log_{10}TCID_{50}$/ml).

e) 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 MNV according to EN 14476:2013 is valid.

7. Results

Results of examination are shown in tables 1 to 8. Tables 1 to 7 demonstrate the raw data, whereas table 8 (a+b) gives a summary of results.

The test product as 1.75 % solution was able to inactivate MNV after 5 minute under dirty conditions in this quantitative suspension test. The reduction factor was ≥ 5.13± 0.29 at this time point (Table 1). This corresponded to an inactivation of ≥ 99.999 %.

Tested as 1.0 % solution, the test product was also active within 5 minutes of exposure time (Table 2). The reduction factor was ≥ 5.13± 0.29.

The 0.5 % solution of the test product was also active within 5 minutes of exposure time (Table 3). The reduction factor was ≥ 5.13 ± 0.29.

Tested as 0.1 % solution, the test product was not active within 30 minutes of exposure time (Table 4).

8. Conclusion

The instrument disinfectant Chemides Pulver tested as 0.5 % solution demonstrated effectiveness against MNV after an exposure time of 5 minute under dirty conditions.
Therefore, the instrument disinfectant Chemides Pulver can be declared as active against MNV as follows:

0.5 % 5 minutes

Bremen, 10.06.2015

- 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 revisedat 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 MNV
Table 2:	Raw data for Chemides Pulver (1.0 %) tested against MNV
Table 3:	Raw data for Chemides Pulver (0.5 %) tested against MNV
Table 4:	Raw data for Chemides Pulver (0.1 %) tested against MNV
Table 5:	Raw data for formaldehyde solution (0.7 %) tested against MNV
Table 6:	Raw data for control of efficacy for suppression of disinfectant's activity (1.75 %)
Table 7:	Raw data (MNV) for cell sensitivity (1.75 %)
Table 8 (a+b):	Summary of results with Chemides Pulver and MNV

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 MNV at 20 °C (quantal test; 8 wells) (3933)

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	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
			10	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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	1.75%	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	4444 4444	0000 0004	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 0444	0404 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 MNV at 20 °C (quantal test; 8 wells) (3933)

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	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
			10	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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	1.0%	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	4444 4444	0000 0004	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 0444	0404 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 MNV at 20 °C (quantal test; 8 wells) (3933)

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.	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	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	4444 4444	0000 0004	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 0444	0404 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 MNV at 20 °C (quantal test; 8 wells) (3933)

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	4444 4444	4444 4404	4440 0444	0000 0400	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	4444 4444	0000 0004	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 0444	0404 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 formaldehyde solution (0.7 %) tested against MNV at 20 °C (quantal test; 8 wells) (3933)

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	tttt tttt	4444 4444	4444 4444	0444 4044	0004 0000	n.d.	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 0400	0000 0400	n.d.	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	4444 4444	0040 4444	0000 0000	0000 0000	n.d.	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	4000 0440	0000 0000	0000 0000	0000 0000	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	tttt tttt	tttt tttt	tttt tttt	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	0400 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 control of efficacy for suppression of disinfectant's activity (1.75 %) (3933)

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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4040 0404	0004 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 7: Raw data (MNV) for cell sensitivity (1.75 %) (3933)

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	0444 0444	0000 0000	0040 0040	n.d.
test product	1:10	tttt tttt	tttt tttt	tttt tttt	tttt tttt	tttt tttt	tttt tttt	tttt tttt	tttt tttt	n.d.
test product	1:100	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 0440	4000 0040	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 8a: Summary of results with Chemides Pulver and MNV

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	1.50	≤1.50±0.00	≤1.50±0.00	n.d.	n.d.	n.d.	5 (RF ≥5.13±0.29)
test product	1.0%	dirty conditions	1.50	≤1.50±0.00	≤1.50±0.00	n.d.	n.d.	n.d.	5 (RF ≥5.13±0.29)
test product	0.5%	dirty conditions	1.50	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	n.d.	5 (RF ≥5.13±0.29)
test product	0.1%	dirty conditions	1.50	n.d.	n.d.	n.d.	4.25 ±0.48	n.d.	> 30

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

Table 8b: Summary of results with Chemides Pulver and MNV

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	4.50	n.d.	7.38±0.41	7.25±0.44	6.13±0.37	4.88±0.37	> 60
virus contr.	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.25	n.a.
virus contr.	n.a.	dirty conditions	n.a.	7.63±0.25	n.d.	n.d.	n.d.	7.63±0.41	n.a.
suppression control	1.75%	dirty conditions	1.50	n.d.	n.d.	n.d.	7.13±0.45	n.d.	n.a.
sens.control PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.46	n.a.
sens. control test product	1.75% → 1:100	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.46	n.a.

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

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

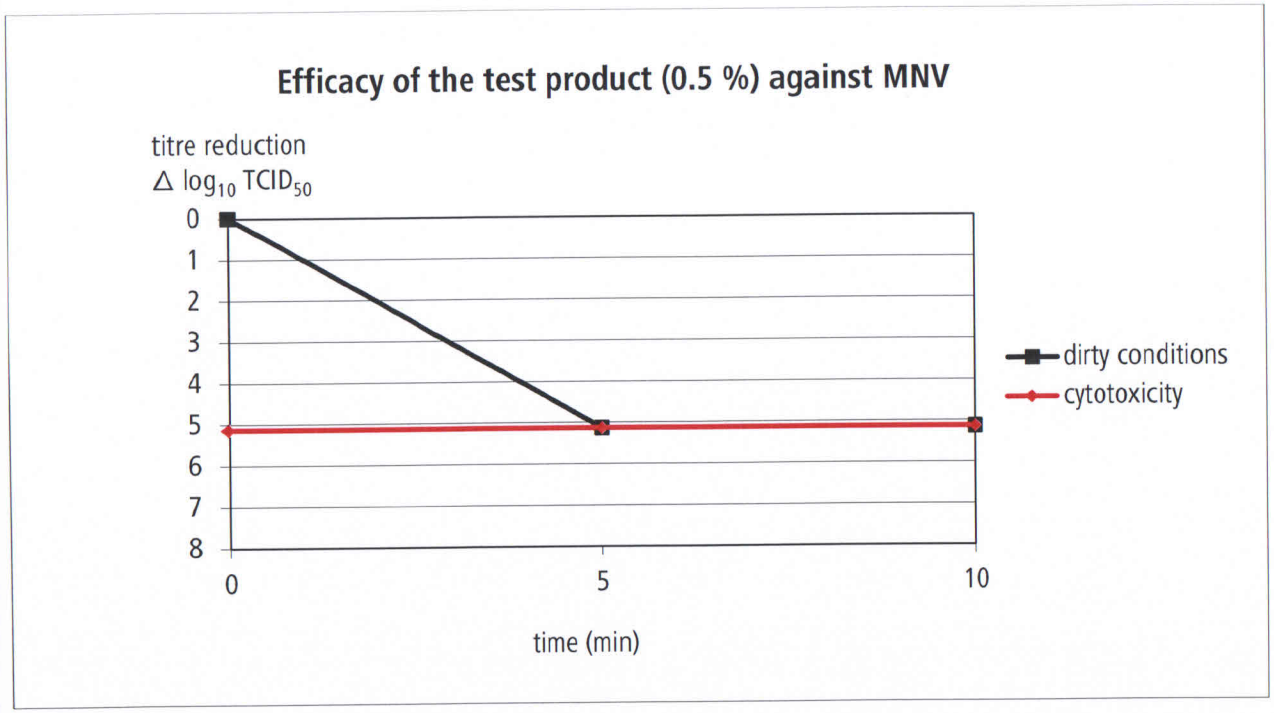

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)