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

TEST REPORT

03/03/2021

Test report L20/2025MV.1

Evaluation of the effectiveness of

UVC BOX

Test virus: modified vaccinia virus Ankara (MVA)

Method: based on EN 16777:2018 (clean conditions)

Chemical disinfectants and antiseptics – Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area – Test method and requirements (phase 2/step 2)

Sponsor:
UVC-Box A/S
Juliesmindevej 15
DK - 4180 Sorø

Norderoog 2, DE - 28259 Bremen
Tel.: +49 40-557631-0, Fax: +49 40-557631-11
info@brillhygiene.com, http://www.brillhygiene.com

1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the device for surface disinfection UVC BOX against modified vaccinia virus Ankara (MVA) using the quantitative carrier test based on EN 16777 (1) under clean conditions.

2. Identification of test laboratory

Dr. Brill + Partner GmbH, Institute for Hygiene and Microbiology, Norderoog 2, DE - 28259 Bremen

3. Identification of device

Manufacturer	UVC-Box A/S
Name of product	UVC BOX
Confirmation no.	219652
Serial number	M2011013
Application	surface disinfection
System	UV light radiation with 6 UVC light sources
Date of arrival in the laboratory	11/12/2020

4. Materials

4.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880120)
- fetal calf serum (Thermo Fisher, article no. CH30160.02)
- Decon 90® (Zinsser Analytic GmbH, article no. 80000)
- Aqua dest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, Cohn-Fraction V, article no. CA-2153)
- glutaraldehyde (AppliChem [2 %], article no. A4316)
- Penicillin/ streptomycin (Sigma-Aldrich, article no. P-0781).

4.2 Virus and cells

The modified vaccinia virus Ankara (MVA) originated from Dr. Manteufel, Institut für Tierhygiene und Öffentliches Veterinärwesen, DE - 04103 Leipzig. Before inactivation assays, virus had been passaged three times in *BHK 21-cells* (Baby Hamster Kidney).

BHK 21-cells (passage 37) originated from the Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, DE - 38124 Braunschweig.

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

- CO_2 incubator
- 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, Germany)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Container, flat bottom, 25 cm, with cap (Sarstedt AG & Co., Nümbrecht, Germany)
- Stainless steel discs (2 cm diameter discs) with Grade 2 B finish on both sides (article no. 4174-3000, GK Formblech GmbH, Berlin, Germany)
- Glass petri dishes (Nunc GmbH & Co. KG, Wiesbaden, Germany).

5. Experimental conditions

Test temperature (range given in the norm)	room temperature (18 °C ± 1 °C to 25 °C ± 1 °C)
Test temperature (measured in the room outside the UVC BOX during test performance)	22.5 °C
Concentration(s) of test product	not applicable
Appearance of product dilution(s)	not applicable
Contact time(s)	3 minutes, gives 300 mj/cm^2 UVC light
Interfering substance(s) in the virus inoculum(s)	clean conditions: 0.3 g/l bovine serum albumin (BSA)
Diluent of the test product	not applicable
Procedure to stop action of disinfectant	immediate dilution with ice-cold cell medium
Test virus	modified vaccinia virus Ankara (MVA) (ATCC VR-1508)
Period of analysis	25/02/2021 – 02/03/2021
End of testing	03/03/2021

6. Methods

6.1 Preparation of test virus suspension

For preparation of test virus suspension, 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 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.

6.2 Preparation of disinfectant (dilutions)

The principle of the UVC BOX is based on UV radiation inside the device. Thus, a preparation is not necessary.

6.3 Preparation of the stainless steel discs

Prior to use the discs shall be placed in a container with an appropriate quantity of 5 % (v/v) Decon 90® for 60 minutes. Subsequently, the discs were rinsed with running freshly demineralised water for 2 x 10 s, dipped in a bath containing 70 % (v/v) propan-2-ol for 15 min and rinsed again with distilled water for at least 3 x 10 seconds. Finally, the discs were sterilised by autoclaving (steam sterilisation) according to 16777, section EN 5.3.3.

6.4 Preparation of the virus inoculum

Nine volumes of test virus suspension were mixed with one volume of interfering substance solution (EN 16777, section 5.2.2.8).

6.5 Inactivation assays and controls

Tests were carried out in based on EN 16777 (1) at room temperature. For each test, 2 discs per test series and exposure time plus control discs were prepared. Three test series were performed.

Test discs were placed aseptically in a Petri dish and inoculated with 50 µl of the virus inoculum (EN 16777, section 5.5.2). After drying, the discs were placed in the middle on the net at the bottom inside the device in a horizontal position. After the appropriate contact time, action of the product was stopped by immediate dilution with 9.9 ml ice-cold medium (1:100 dilution) in a separate container (25 cm with cap). Container was vortexed for 60 seconds to re-suspend the virus. Directly after elution, series of ten-fold dilutions of the eluate in ice-cold maintenance medium were prepared and inoculated on cell culture (see EN 16777, section 5.5.2).

Titrations of the virus controls were performed before drying, directly after drying (VCt0) and after drying at the respective contact time(s).

In addition, a control of efficiency for suppression of disinfectant's activity was included (EN 16777, section 5.5.4).

For the control of cell susceptibility (EN 16777, section 5.5.3.2) one volume of the lowest apparently non-cytotoxic dilution of the eluate (or PBS as control) was added to one volume of double concentrated cell suspension. After 1 h at 37 °C the cells were centrifuged and re-suspended in cell culture medium.
Finally, a comparative titration of the test virus suspension with the virus inoculums was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

Determination of cytotoxicity was performed as described in EN 16777, section 5.5.3.1. Stainless steel discs were inoculated with 45 µl cell culture medium (with FCS) and 5 µl interfering substance instead of the virus inoculum.

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

As reference for test validation according to EN 16777, section 5.5.5 glutaraldehyde (50 ppm) was included. 5 minutes were chosen as contact time.

6.6 Determination of infectivity

Infectivity was determined by means of end point dilution method according to EN 16777, section 5.5.2 by transferring 0.1 ml of each dilution into eight wells of a microtitre plate containing 0.1 ml freshly splitted cells per well, beginning with the highest dilution. This cell suspension was adjusted to reach 10-15 x 10^3 cells per well. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3).

6.7 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the reduction of virus titre of dried virus inoculum after treatment with the disinfectant in comparison with the virus control titration treated with water. The difference is given as reduction factor (RF).

According to EN 16777, section 5.8.2, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if within the recommended exposure period the titre is reduced at least by 4 $\log_{10}$ steps.

7. Results

7.1 Determination of cytotoxicity

In parallel with the inactivation tests, cytotoxicity of the eluates after test performance and of 50 ppm glutaraldehyde was measured.

The glutaraldehyde solution (50 ppm) showed no cytotoxicity in the first dilution. This corresponded to a $\log_{10}$ CD_{50}/ml of 1.50 (table 7).
Examinations also showed that the eluates were not cytotoxic in the first dilution. This corresponded to a $\log_{10}$ CD_{50}/ml of 1.50.

These tests to measure cytotoxicity are imperative, because in this manner the lower detection threshold for non-inactivated MVA could be determined.

7.2 Control of efficiency for suppression of disinfectant's (disinfection system´s) activity

The logarithmic titre of this virus control without drying was 6.63 ± 0.41 versus 6.38 ± 0.41 log_{10} $TCID_{50}$/ml for the disinfection system (tables 4 and 7). As prescribed in the EN 16777 (section 5.5.4.1), the difference of the logarithmic titre of the control for suppression of disinfectant's activity was ≤ 0.5 log_{10} compared to the virus inoculum.

7.3 Control of cell sensitivity

A non-cytotoxic concentration of the disinfectant might inhibit the virus replication. Therefore, cell sensitivity in a non-cytotoxic concentration was evaluated by a comparative titration.

This comparative virus titration on cells treated with the eluate of the inactivation assay (virus titre: 6.50 ± 0.00 log_{10} $TCID_{50}$/ml) or PBS (virus titre: 6.88 ± 0.37 log_{10} $TCID_{50}$/ml) resulted in a difference of < 1.0 log_{10} demonstrating that virus replication was not inhibited (tables 5 and 7).

7.4 Virus-inactivating properties of reference control(s)

Results of inactivation tests are found in tables 3 and 7 as well as in figure 2. Glutaraldehyde (50 ppm) reduced the virus titre after 5 minutes by 0.69 ± 0.55 log_{10} steps. Here, the RF of glutaraldehyde was in the range of the values given in EN 16777, section 5.7 table 3 (< 3 log).

7.5 Virus-inactivating properties of disinfectant

The surface disinfection device UVC BOX was tested with an exposure time of 3 minutes with each two parallels in three test series.

Results of examinations are shown in tables 1 to 7. Tables 1 to 5 demonstrate the raw data, whereas tables 6 and 7 give a summary of results.

The UVC BOX was able to inactivate MVA after 3 minutes exposure time under clean conditions in the quantitative carrier test. The average reduction factor and its 95 % confidence interval achieved ≥ 4.63 ± 0.36 at this time point (tables 2 and 6). This corresponded to an inactivation of ≥ 99.99 %.

8. Verification of the methodology

The following criteria as mentioned in the EN 16777, section 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of ≥ 4 $\log_{10}$ reduction.

b) The eluate of the inactivation assay shows no cytotoxicity in the 1:100 dilution thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre.

c) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test with glutaraldehyde (EN 16777) was in the range of the values specified in the EN 16777 (section 5.7, table 3).

d) The cytotoxicity of the product solution does not affect cell morphology and cell growth or the susceptibility of the test organism in the dilutions of the test product used for demonstration of a 4 log reduction.

e) The comparative titration on pre-treated (eluate disinfection system) and non-pre-treated (PBS) cells showed an acceptable difference (< 1.0 $\log_{10}$; EN 16777) of virus titre.

f) The difference of the logarithmic titre of the control for suppression of disinfectant's (disinfection system´s) activity was in the range to the specification of the norm (≦ 0.5 $\log_{10}$, EN 16777) compared to the virus control before drying.

Since all criteria according to EN 16777, section 5.7 were fulfilled, examination with MVA based on EN 16777 was valid.

9. Conclusion

The surface disinfection device UVC BOX demonstrated effectiveness against MVA after an exposure time of 3 minutes under clean conditions. Therefore, UVC BOX can be declared as effective against MVA under clean conditions in the test simulating practical conditions (based on EN 16777) as follows:

UV radiation for 3 minutes **clean conditions**

Bremen, 03/03/2021

- Dr. Britta Becker -
Head of Laboratory

- Dr. Dajana Paulmann -
Scientific Project Manager

10. Literature

1. EN 16777: Chemical disinfectants and antiseptics – Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area – Test method and requirements (phase 2, step 2), German version 2018.

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 of the virus controls

Table 2: Raw data of UVC BOX tested against MVA

Table 3: Raw data of the reference control(s) tested against MVA

Table 4: Raw data of the control of efficacy for suppression of disinfectant's activity

Table 5: Raw data (MVA) for cell sensitivity to virus

Table 6: Results with UVC BOX and MVA (summary 1)

Table 7: Results with UVC BOX and MVA (summary 2)

Legend to the figures

Figure 1: Virus-inactivating properties of UVC BOX

Figure 2: Virus-inactivating properties of reference 1

Table 1: Raw data of the virus controls (quantal test; 8 wells) (#7178)

Product	Concentration	Interfering substance	Contact time (min)	Carrier	Dilutions (log_{10})								
					1	2	3	4	5	6	7	8	9
virus control before drying (virus inoculum)	n.a.	clean	n.a.	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4423 0444	3000 4000	0000 0000	0000 0000	0000 0000
virus control after drying	n.a.	clean	0	1	4444 4444	4444 4444	4444 4444	4444 4444	4000 0400	0000 0000	0000 0000	0000 0000	0000 0000
				2	4444 4444	4444 4444	4444 4444	4444 4444	4020 4300	0000 0000	0000 0000	0000 0000	0000 0000
virus control after drying	n.a.	clean	3	1	4444 4444	4444 4444	4444 4444	4444 4444	4032 0444	0000 0000	0000 0000	0000 0000	0000 0000
				2	4444 4444	4444 4444	4444 4444	4444 4444	4402 0003	0000 0000	0000 0000	0000 0000	0000 0000
virus control after drying	n.a.	clean	5	1	4444 4444	4444 4444	4444 4444	4444 4444	4440 0344	0000 0034	0000 0000	0000 0000	0000 0000
				2	4444 4444	4444 4444	4444 4444	4444 4444	4440 4234	0000 0020	0000 0000	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 2: Raw data of UVC BOX tested against MVA (quantal test; 8 wells) (#7178)

Product	Test series	Interfering substance	Contact time (min)	Carrier	Dilutions (log_{10})								
					1	2	3	4	5	6	7	8	9
UVC BOX	1	clean	3	1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
				2	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
UVC BOX	2	clean	3	1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
				2	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
UVC BOX	3	clean	3	1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
				2	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
product cytotoxicity	n.a.	clean	3	1	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.	n.d.

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

0 = no virus present
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

t = cytotoxic

Table 3: Raw data of the reference control(s) tested against MVA (quantal test; 8 wells) (#7178)

Product	Concentration	Interfering substance	Contact time (min)	Carrier	Dilutions (log_{10})								
					1	2	3	4	5	6	7	8	9
50 ppm glutaraldehyde	undiluted	clean	5	1	4444 4444	4444 4444	4444 4444	4442 4342	0000 2000	0000 0003	0000 0000	n.d.	n.d.
				2	4444 4444	4444 4444	4444 4444	4444 4444	0004 3004	0000 0000	0000 0000	n.d.	n.d.
50 ppm glutaraldehyde cytotoxicity	undiluted	clean	5	1	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.	n.d.

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 4: Raw data of the control of efficacy for suppression of disinfectant's (disinfection system´s) activity (#7178)

Product	Concentration	Interfering substance	Eluate dilution	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
UVC BOX	n.a.	clean	1:100* (= 10^{-1})	n.d.	4444 4444	4444 4444	4444 4444	4403 0444	3000 0000	0000 0000	0000 0000	n.d
virus control	n.a.	clean	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4423 0444	3000 4000	0000 0000	0000 0000	0000 0000

*Data refer to the dilution of the test substance after termination of the action of the product by immediate dilution. In the 1:10 dilution reaction was stopped in 900 µL medium (undiluted eluate =10^{-0}), in the 1:100 dilution (=10^{-1}) reaction was stopped in 9.9 ml medium

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 5: Raw data (MVA) for cell sensitivity to virus (#7178)

Product	Concentration	Disinfectant dilution (Eluate dilution)	Dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
UVC BOX	n.a.	1:100 (= 10^{-1})	4444 4444	4444 4444	4444 4444	4444 4444	4424 4344	0000 0000	0000 0000	0000 0000	n.d.
PBS	n.a.	1:100 (= 10^{-1})	4444 4444	4444 4444	4444 4444	4444 4444	4444 4433	2001 0040	0000 0000	0000 0000	n.d.

*Data refer to the dilution of the test substance after termination of the action of the product by immediate dilution. In the 1:10 dilution reaction was stopped in 900 µL medium (undiluted eluate =10^{-0}), in the 1:100 dilution (=10^{-1}) reaction was stopped in 9.9 ml medium

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 6: Results with UVC BOX and MVA (summary 1)

	Interfering substance in virus inoculum	log_{10} $TCID_{50}$/ml with 95% CI	log_{10} $TCID_{50}$/ml with 95% CI after drying									reduction	
		before drying	carrier 1	carrier 2	carrier 3	carrier 4	carrier 5	carrier 6	MV	mean 95 % CI	2xSD[1]	RF	95 % CI
VC (virus inoculum)	clean	6.63±0.41	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	**6.63**	**0.41**	**n.a.**	**n.a.**	**n.a.**
VCt0	clean	n.a.	5.75±0.33	6.00±0.38	n.d.	n.d.	n.d.	n.d.	**5.88**	**0.36**	**0.35**	**0.75**	**0.55**
VCt3	clean	n.a.	6.25±0.33	6.00±0.38	n.d.	n.d.	n.d.	n.d.	**6.13**	**0.36**	**0.35**	**0.50**	**0.55**
UVC BOX t3 min	clean	n.a.	≤1.50±0.00	≤1.50±0.00	≤1.50±0.00	≤1.50±0.00	≤1.50±0.00	≤1.50±0.00	**≤1.50**	**0.00**	**0.00**	**≥4.63**	**0.36**

[1] - This value shows the spread of the parallel approaches

n.a. = not applicable
n.d. = not done
CI = confidence interval
SD = standard deviation
RF = reduction factor
VC = virus control
MV = mean value

Table 7: Results with UVC BOX and MVA (summary 2)

	Interfering substance in virus inoculum	log_{10} $TCID_{50}$/ml with 95% CI	log_{10} $TCID_{50}$/ml with 95 % CI after drying					reduction	
		before drying	carrier 1	carrier 2	MV	mean 95 % CI	2xSD	RF	95 % CI
VC (virus inoculum)	clean	6.63±0.41	n.a.	n.a.	**6.63**	**0.41**	**n.a.**	**n.a.**	**n.a.**
VCt5	clean	n.a.	6.50±0.46	6.50±0.35	**6.50**	**0.41**	**0.00**	**0.13**	**0.58**
50 ppm glutaraldehyde t5 min	clean	n.a.	5.75±0.35	5.88±0.37	**5.81**	**0.36**	**0.18**	**0.69**	**0.55**
suppression of disinfection (1:10)	clean	n.d.	n.a.	n.a.	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**
suppression of disinfection (1:100)	clean	6.38±0.41	n.a.	n.a.	**n.a.**	**n.a.**	**n.a.**	**0.25**	**n.a.**
PBS control	clean	6.88±0.37	n.a.	n.a.	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**
cell sensitivity (1:100)	clean	6.50±0.00	n.a.	n.a.	**n.a.**	**n.a.**	**n.a.**	**0.38**	**n.a.**

n.a. = not applicable CI = confidence interval RF = reduction factor MV = mean value
n.d. = not done SD = standard deviation VC = virus control

Figure 1: Virus-inactivating properties of UVC BOX

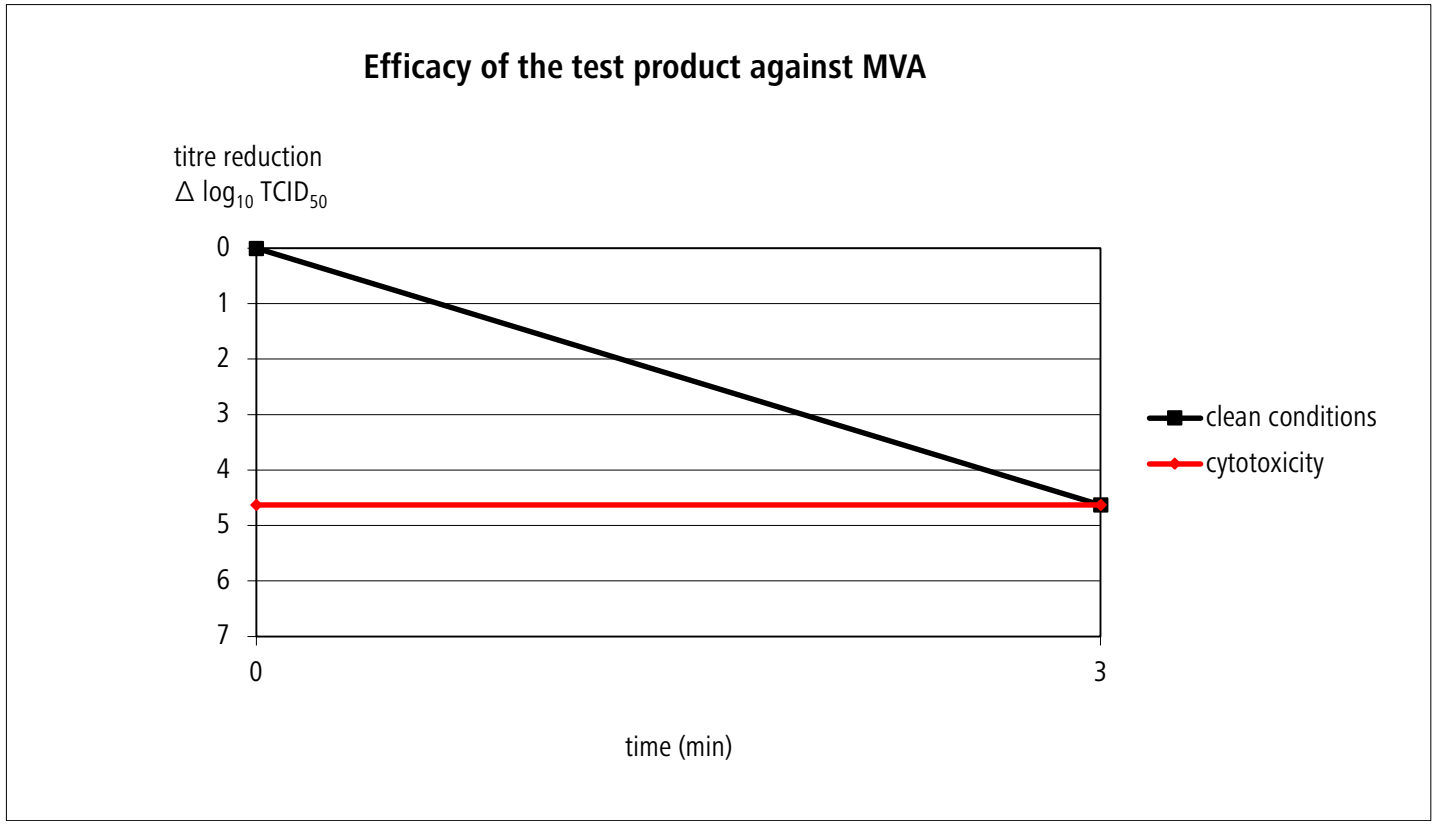

Figure 2: Virus-inactivating properties of reference (glutaraldehyde)