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TEST REPORT

15/06/2020

Test report L20/0542BC.1

Evaluation of the effectiveness of

GVK Geliksi GeveDesi Pro

Test virus: bovine coronavirus (BCoV) (surrogate of human coronaviruses)

Method: EN 14476:2013+A2:2019 (clean conditions)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine (phase 2/ step 1)

Sponsor:
GVK Coating Technology Oy
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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

Sponsor	GVK Coating Technology Oy
Name of product	GVK Geliksi GeveDesi Pro
Confirmation no.	214214
Product diluent recommended by the manufacturer	-
Batch number	2000126
Application	hand disinfection
Production date	-
Expiry date	-
Active compound (s) (100 g)	ethanol, 70%
Appearance, odour	Clear, colorless liquid product specific
pH-values	undiluted: 9.41 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	30/04/2020

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880121)
- fetal calf serum (Thermo Fisher, article no. CH30160.02)
- 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).

3.2 Virus and cells

The bovine coronavirus strain L9 was obtained by Dr. G. Zimmer, Institute of Virology at the School of Veterinary Medicine Hannover (Tierärztliche Hochschule, DE - 30559 Hannover).

The *U373 cells* (passage 12) were as well obtained by Dr. G. Zimmer, Institute of Virology at the School of Veterinary Medicine Hannover (Tierärztliche Hochschule, DE - 30559 Hannover).

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.

3.3 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)
- 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	undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	30 seconds and 30 minutes
Interfering substance	0.3 g/l bovine serum albumin (clean conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	Aqua bidest.
Stability of product in the mix with virus and interfering substance (80.0 % solution)	minor clouding, no precipitation
Virus strain	bovine coronavirus strain L9
Date of testing	07/05/2020 – 15/06/2020
End of testing	15/06/2020

5. Methods

5.1 Preparation of test virus suspension

To prepare the test virus suspension, *U373* cells were cultivated in a 175 cm^2 flask with in EMEM supplemented with L-glutamine, non-essential amino acids and sodium pyruvate and 10 % fetal calf serum. Before virus infection, cells were washed two times with phosphate buffered saline (PBS), incubated for 3 h with EMEM without FCS and were washed once with EMEM supplemented with trypsin. For virus production, BCoV strain L9 was added to the prepared monolayer. After an incubation period of 24 to 48 hours (cells showed a constant cytopathic effect), cells were lysed by a rapid freeze/thaw cycle. Cellular debris was removed by low speed centrifugation. After aliquotation of the supernatant, test virus suspension was stored at –80 °C.

5.2 Preparation of disinfectant (dilutions)

The test product was evaluated undiluted. Due to the addition of interfering substance and test virus suspension an 80.0 % solution resulted.

Furthermore, the product was evaluated as 50.0 % and 10.0 % solutions (demonstrating of non-active range). These solutions were prepared with Aqua bidest. immediately before the inactivation tests.

5.3 Infectivity assay

Infectivity was determined by means of end point dilution titration using the microtitre process. For this, samples were immediately diluted at the end of the exposure time with ice-cold EMEM with trypsin and 100 µl of each dilution were placed in eight wells of a sterile polystyrene flat bottomed plate with a preformed *U373* monolayer. Before addition of virus, cells were washed twice with EMEM and incubated for 3 h with 100 µl EMEM with trypsin. Incubation was at 37 °C in a CO_2-atmosphere (5.0 % CO_2 - content). Finally, cultures were observed for cytopathic effects for six days of inoculation. The infectious dose ($TCID_{50}$) was calculated according to the method of Spearman (2) and Kärber (3).

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 4 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 according to EN 5.5. The test product was examined undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions in Aqua bidest. at 20 °C according to EN 14476. 30 seconds and 30 minutes were chosen as contact time.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8}.

Titrations 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 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 °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 the wells of the microtitre plates with a preformed monolayer of *U373 cells*. After at least one hour, a comparative virus titration was performed on the cells treated in such a manner or treated with PBS only.

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 based on 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 ≥ 5.63 ± 0.45)

b) The test product (80.0 %) showed no 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) *U373 cells* showed no significant difference (< 1 log_{10}; EN 5.7) of virus titre: 7.38 ± 0.53 (PBS) versus 7.13 ± 0.49 (1:10 dilutions of disinfectant as 80.0 % solution) log_{10} $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant's activity (80.0 %) showed no decrease (≤ 0.5 log_{10}; EN 5.5.5.1) in virus titre (6.88 ± 0.37 versus 7.13 ± 0.45 log_{10} $TCID_{50}$/ml).

Since these criteria according EN 5.7 were fulfilled, examination with bovine coronavirus according to EN 14476 is valid.

7. Results

Results of examination are shown in tables 1 to 7. Tables 1 to 6 demonstrate the raw data, whereas tables 7 (a+b) give a summary of results.

The undiluted test product in an 80.0 % assay was able to inactivate bovine coronavirus after 30 seconds of exposure time under clean conditions (table 1). The reduction factor was ≥ 5.63 ± 0.45. This corresponded to an inactivation of ≥ 99.999 %.

The test product as 50.0 % was also able to inactivate bovine coronavirus after 30 seconds of exposure time under clean conditions (table 2). The reduction factor was ≥ 5.63 ± 0.45. This corresponded to an inactivation of ≥ 99.999 %.

The test product as 10.0 % solution was not able to inactivate bovine coronavirus within 30 minutes of exposure time under clean conditions (table 3).

8. Conclusion

The hand disinfectant GVK Geliksi GeveDesi Pro tested undiluted demonstrated activity against bovine coronavirus after an exposure time of 30 seconds under clean conditions. Therefore, the hand disinfectant GVK Geliksi GeveDesi Pro can be declared as active against bovine coronavirus as follows:

undiluted **30 seconds** **clean conditions**

Bremen, 15/06/2020

- Dr. Britta Becker -
Head of Laboratory

- Dr. Dajana Paulmann -
Scientific Project Manager

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 revised at 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+A2:2019: 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

Legend to the Figures

Table 1: Raw data for GVK Geliksi GeveDesi Pro (80.0 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6607)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	clean conditions	0.5	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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.
			15	n.a.	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.
test product cytotoxicity	80.0 %	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 4440	0000 0004	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 4040	0000 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 2: Raw data for GVK Geliksi GeveDesi Pro (50.0 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6607)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	50.0 %	clean conditions	0.5	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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.
			15	n.a.	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.
test product cytotoxicity	50.0 %	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 4440	0000 0004	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 4040	0000 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 for GVK Geliksi GeveDesi Pro (10.0 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6607)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				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.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	4444 4444	4444 4444	4444 4444	4444 4444	4400 4444	0000 0000	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 4440	0000 0004	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 4040	0000 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 4: Raw data for formaldehyde solution (0.7 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6607)

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 0444	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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 0444	0000 0000	0000 0000	0000 0000

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

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

Table 5: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %) (#6607)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	clean conditions	n.d.	4444 4444	4444 4444	4444 4444	4444 4444	4304 0000	0000 4444	0000 0000	n.d.
corresponding virus control	clean conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 4040	0000 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 6: Raw data (bovine coronavirus) for cell sensitivity (80.0 %) (#6607)

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	4044 0400	4040 0300	0000 0000	n.d.
test product	1:10	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0403 0400	4000 0040	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 7a: Summary of results with GVK Geliksi GeveDesi Pro and bovine coronavirus

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after …min
				0.5	2	15	30	60	
test product	80.0 %	clean conditions	1.50	≤1.50±0.00	n.d.	n.d.	n.d.	n.d.	0.5 (RF ≥ 5.63±0.45)
test product	50.0 %	clean conditions	1.50	≤1.50±0.00	n.d.	n.d.	n.d.	n.d.	0.5 (RF ≥ 5.63±0.45)
test product	10.0 %	clean conditions	1.50	n.d.	n.d.	n.d.	7.25±0.33	n.d.	> 30 (RF = 0.00±0.56)

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

Table 7b: Summary of results with GVK Geliksi GeveDesi Pro and bovine coronavirus

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.	≤5.63±0.45	≤4.50±0.00	≤4.50±0.00	≤4.50±0.00	≥ 15 (RF ≥ 2.88±0.25)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.38±0.25	n.a.
virus control (+ suppression)	n.a.	clean conditions	n.a.	7.50±0.35	n.d.	n.d.	n.d.	7.13±0.45	n.a.
suppression control	80.0 %	clean conditions	1.50	n.d.	n.d.	n.d.	6.88±0.37	n.d.	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.38±0.53	n.a.
sens. product	80.0 % → 1:10	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.13±0.49	n.a.

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

Figure 1: Virus-inactivating properties of GVK Geliksi GeveDesi Pro (80.0 %)

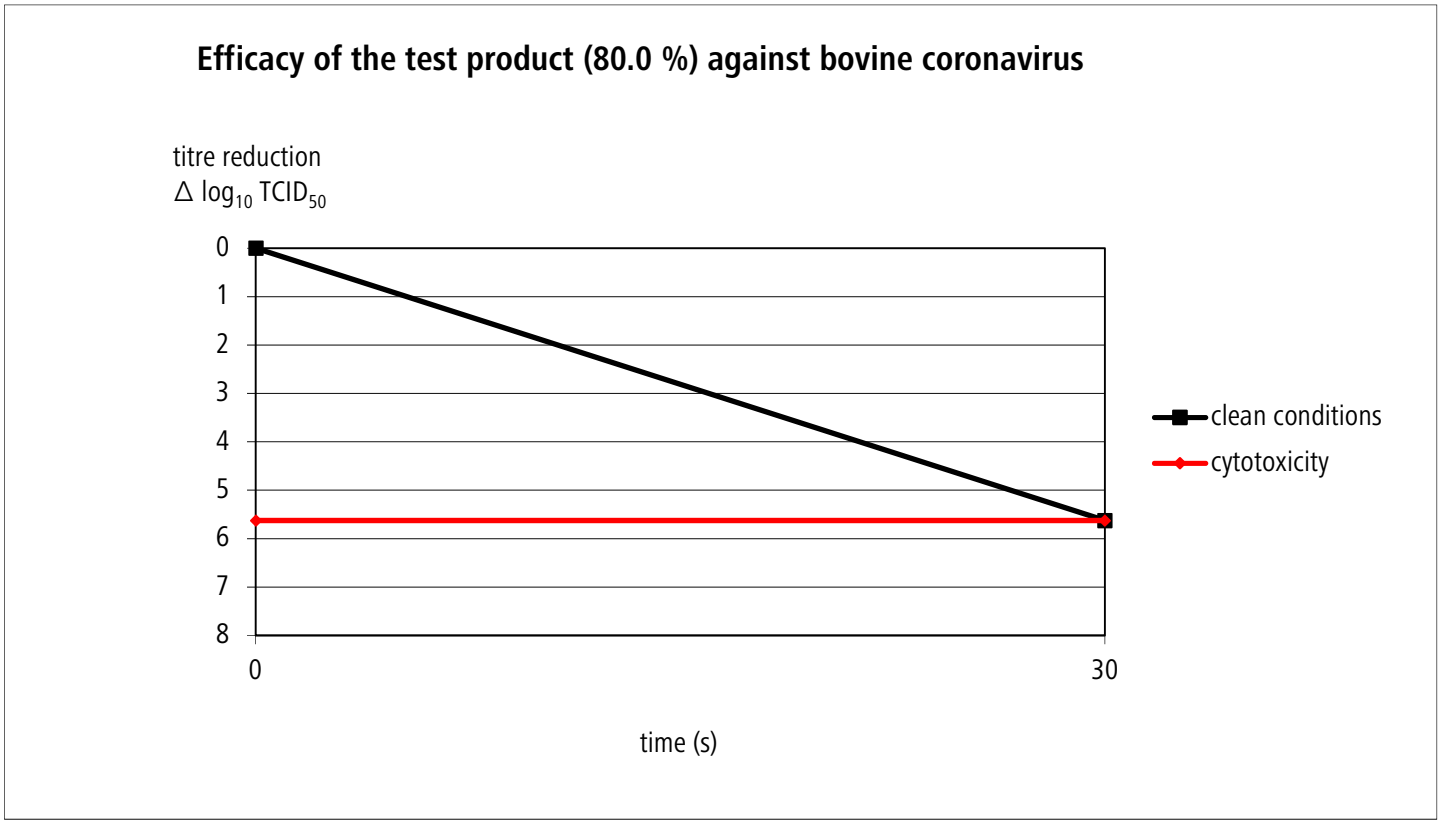

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)