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Mitry-Mory, 2020 September 29th

DECLARATION

Considering the report of the laboratory Dr Brill + Dr Steinmann L20/0407aBC.1 of 02/06/2020 concerning the test EN14476+A2 :2019 on the product PE14.02 5 containing 63% (w/w) ethanol, and presenting a reduction greater than or equal to 4 log on the bovine coronavirus strain L9 at concentrations of 80% and 50% in 30 seconds in a valid test, I can certify, in view of the efficacy of this product diluted at 50% on this virus and of the commonly accepted data on the efficacy of ethanol on coronaviruses1 that :

The equivalent formula PE14.02 6, named Purell Advanced Hygienic Hand rub, containing 63.17% (w/w) ethanol and denatured with 3.41% isopropanol should show a reduction of more than 4 log on bovine coronavirus strain L9 at the concentration of 80% in 30 seconds of contact according to EN14476+A2 :2019.

Philippe STROHL
Veterinary Doctor
Scientific Director Europe

[1] par exemple :
Inactivation of Severe Acute Respiratory Syndrome Coronavirus 2 by WHO-Recommended Hand Rub Formulations and Alcohols
Annika Kratzel et al. EID Jurnal Vol. 26 N°7 July 2020 - Figure 1

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

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ZLG-AP-216.11.02

TEST REPORT

02/06/2020

Test report L20/0407aBC.1

Evaluation of the effectiveness of

PE14.02 5

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:
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a GOJO Family Company
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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	Laboratoires Prodene Klint a GOJO Family Company
Name of product	PE14.02 5
Confirmation no.	214789
Product diluent recommended by the manufacturer	-
Batch number	069.20
Application	hand disinfection
Production date	-
Expiry date	-
Active compound (s) (100 g)	63 % (w/w) ethanol
Appearance, odour	clear, colorless gel product specific
pH-values	undiluted: 7.33 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	14/04/2020

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)
- Trypsin (SERVA Electrophoresis GmbH, article no. 37290).

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 11) 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)	medium clouding, no precipitation
Virus strain	bovine coronavirus strain L9
Date of testing	29/04/2020 – 02/06/2020
End of testing	02/06/2020

5. Methods

5.1 Preparation of test virus suspension

For preparation of test virus suspension, *U373* cells were cultivated in a 175 cm2 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 test virus suspension and interfering substance an 80.0 % solution resulted.

Furthermore, the product was evaluated as 50.0 % and 10.0 % solutions (demonstration 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 CO2-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 times.

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 ≥ 4.00 ± 0.35)

b) The test product (80.0 %) showed 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: 6.63 ± 0.25 (PBS) versus 6.00 ± 0.38 (1:100 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 (6.13 ± 0.37 versus 6.50 ± 0.35 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 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 table 7 (a+b) gives 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 ≥ 4.00 ± 0.35. This corresponded to an inactivation of ≥ 99.99 %.

The test product as 50.0 % solution was also able to inactivate bovine coronavirus after 30 seconds of exposure time under clean conditions (table 2). The reduction factor was ≥ 4.00 ± 0.35. This corresponded to an inactivation of ≥ 99.99 %.

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

8. Conclusion

The hand disinfectant PE14.02 5 tested undiluted demonstrated activity against bovine coronavirus after an exposure time of 30 seconds under clean conditions. Therefore, the hand disinfectant PE14.02 5 can be declared as active against bovine coronavirus as follows:

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

Bremen, 02/06/2020

- 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

Table 1: Raw data for PE14.02 5 (80.0 %) tested against bovine coronavirus

Table 2: Raw data for PE14.02 5 (50.0 %) tested against bovine coronavirus

Table 3: Raw data for PE14.02 5 (10.0 %) tested against bovine coronavirus

Table 4: Raw data for formaldehyde solution (0.7 %) tested against bovine coronavirus

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

Table 6: Raw data (bovine coronavirus) for cell sensitivity (80.0 %)

Table 7 (a+b): Summary of results with PE14.02 5 and bovine coronavirus

Legend to the Figures

Figure 1: Virus-inactivating properties of PE14.02 5 (80.0 %)

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

Table 1: Raw data for PE14.02 5 (80.0 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6591)

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	tttt tttt	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.
			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.	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	4004 0000	0040 0000	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	0444 4444	0200 0000	0000 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 2: Raw data for PE14.02 5 (50.0 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6591)

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	n.d.	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.
			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	4004 0000	0040 0000	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	0444 4444	0200 0000	0000 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 3: Raw data for PE14.02 5 (10.0 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6591)

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	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.
			5	n.d.	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	4404 0444	0000 0000	0000 0000	n.d.	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	4004 0000	0040 0000	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	0444 4444	0200 0000	0000 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 4: Raw data for formaldehyde solution (0.7 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6591)

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	0000 4040	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	0444 4420	4000 4000	0000 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 5: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %) (#6591)

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	0004 4444	0000 0000	0000 0000	0000 0000	n.d.
corresponding virus control	clean conditions	4444 4444	4444 4444	4444 4444	4444 4444	0444 4444	0200 0000	0000 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 6: Raw data (bovine coronavirus) for cell sensitivity (80.0 % solution) (#6591)

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	4000 0000	0000 0000	0000 0000	n.d.
test product	1:100	4444 4444	4444 4444	4444 4444	4444 4444	4000 4044	0000 0000	0000 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 7a: Summary of results with PE14.02 5 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	5	30	60	
test product	80.0 %	clean conditions	2.50	≤ 2.50±0.00	n.d.	n.d.	n.d.	n.d.	0.5 (RF ≥ 4.00±0.35)
test product	50.0 %	clean conditions	2.50	≤ 2.50±0.00	n.d.	n.d.	n.d.	n.d.	0.5 (RF ≥ 4.00±0.35)
test product	10.0 %	clean conditions	1.50	n.d.	n.d.	n.d.	6.25±0.33	n.d.	> 30 (RF = 0.25±0.48)

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

Table 7b: Summary of results with PE14.02 5 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.	≤ 4.75±0.33	≤ 4.50±0.00	≤ 4.50±0.00	≤ 4.50±0.00	≥ 15 (RF ≥ 2.00±0.46)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	6.50±0.46	n.a.
virus control (+ suppression)	n.a.	clean conditions	n.a.	6.88±0.41	n.d.	n.d.	n.d.	6.50±0.35	n.a.
suppression control	80.0 %	clean conditions	2.50	n.d.	n.d.	n.d.	6.13±0.37	n.d.	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.25	n.a.
sens. product	80.0 % → 1:100	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.00±0.38	n.a.

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

Figure 1: Virus-inactivating properties of PE14.02 5 (80.0 %)

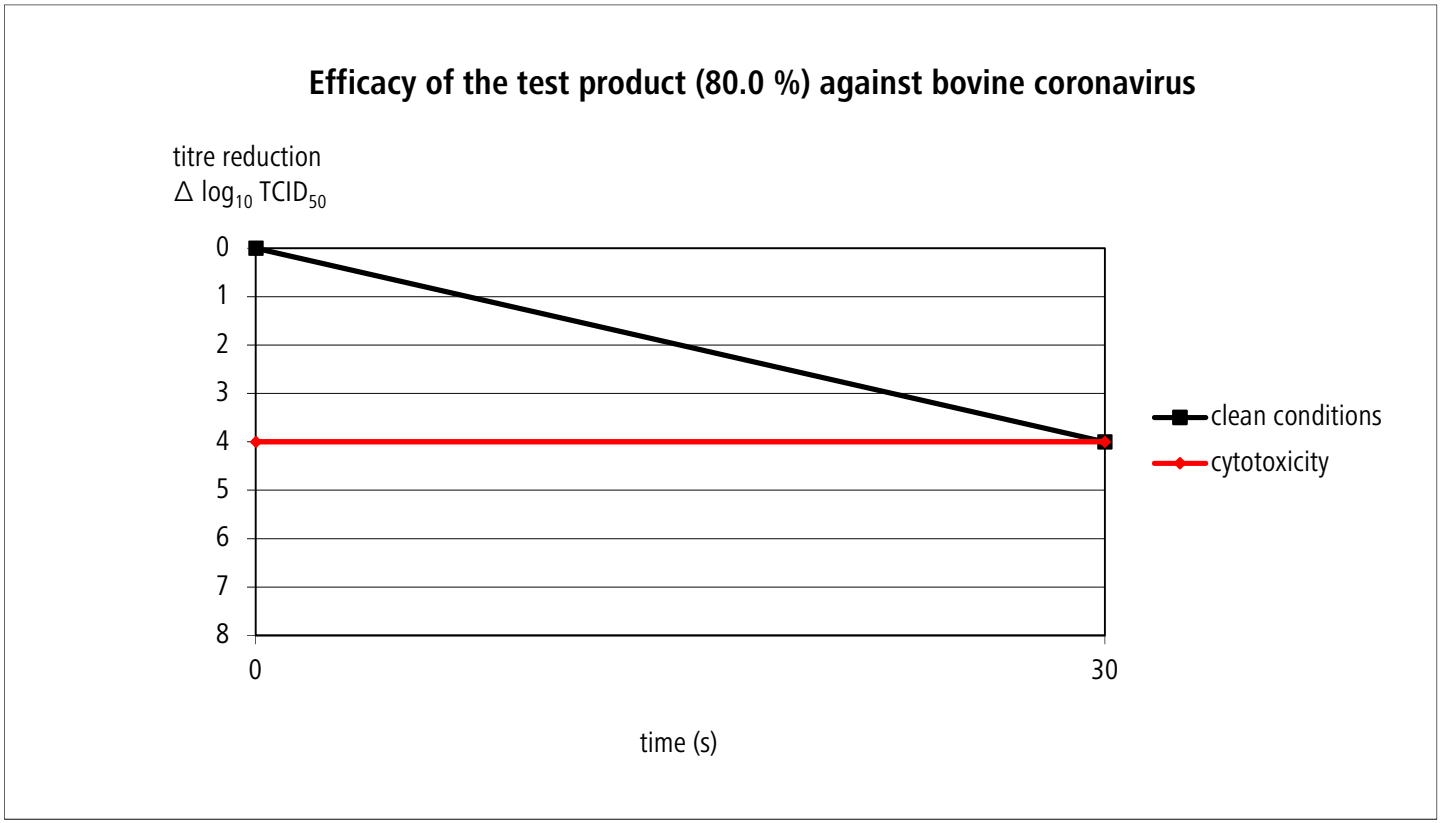

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)