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TEST REPORT

07/11/2020

Test report L20/0285BC.4

Evaluation of the effectiveness of

BARRIER TECH MF Sanitiser Concentrate

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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1. Identification of test laboratory

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

2. Identification of sample

Manufacturer	Teknologisk Institut
Name of product	BARRIER TECH MF Sanitiser Concentrate
Confirmation no.	217044
Product diluent recommended by the manufacturer	-
Batch number	920810-1
Application	surface disinfection
Production date	-
Expiry date	-
Active compound (s) (100 g)	dodecyl-dimethyl ammonium chloride CAS No. 7173-51-5, 15% Alkyl (C12-16), di-methyl benzyl ammonium chloride, CAS No. 68424-85-1, 10%
Appearance, odour	clear, light blue liquid product specific
pH-values	undiluted: 6.51 (20 °C) 0.5 %: 7.32 (20°) 0.25 %: 7.36 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	20/03/2020

3. Materials

3.1 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)
- 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 16) 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	0.5 %, 0.25 % and 0.05 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	5 minutes
Interfering substance	0.3 g/l bovine serum albumin (clean conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water of standardized hardness (WSH)
Stability of product in the mix with virus and interfering substance (0.5 % solution)	no clouding, no precipitation
Virus strain	bovine coronavirus strain L9
Date of testing	08/07/2020 – 27/10/2020
End of testing	07/11/2020

5. Methods

5.1 Preparation of test virus suspension

For preparation of 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 tested as 0.5 %, 0.25 % and 0.05 % solutions under dirty conditions (1 part test virus suspension + 1 part interfering substance + 8 parts disinfectant). Due to the addition of interfering substance and test virus suspension the solutions had to be prepared by the factor 1.25.

These solutions were prepared with WSH 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 (end point titration)

Determination of virucidal activity has been carried out according to EN 5.5.

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 Inactivation assay following the large volume plating method (LVP)

Following the large volume plating method (EN 5.5.4.3) the inactivation assays were further diluted 1:1,000 in cell culture medium. The total volume was added (without any further dilution) to the permissive cells. By introducing such a huge dilution it is possible to eliminate cytotoxicity of the test product in order to demonstrate a 4 $\log_{10}$ reduction of virus titre. Calculation of virus titre follows formula of Taylor or Poisson (EN B.3). This method is necessary for those products which demonstrate a great cytotoxicity.

62.5 µl of the inactivation assay were added to 62.5 ml medium and then the total volume was distributed in 6 microtitre plates (108 µl / well, 576 wells total). After 6 days of inoculation cultures were observed for cytopathic effects.

5.7 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.8 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.

5.9 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).

5.10 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 ≥ 5.10 ± 0.38, LVP)

b) The test product (0.5 %) showed cytotoxicity in the 1:100 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) cells showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 7.00 ± 0.38 (PBS, LVP) versus 6.75 ± 0.33 (1:1,000 dilutions of disinfectant as 0.5 % solution, LVP) $\log_{10}$ $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant's activity (0.5 %) showed a decrease of 1.38 (5.63 ± 0.25 versus 7.00 ± 0.38 $\log_{10}$ $TCID_{50}$/ml) and failed the requirement of the EN (≤ 0.5 $\log_{10}$; EN 5.8). In these experiments at the end of the defined exposure time the test mixture was immediately diluted not 1:10 as described in the control of efficacy for suppression of disinfectant's activity but directly 1:1,000 (LVP) and the dilutions transferred to the cell culture. For this reason, this control is not relevant when using the LVP. Therefore, despite the insufficient control of efficacy for suppression of disinfectant's activity the assay is valid.

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 10. Tables 1 to 8 demonstrate the raw data, whereas tables 9 (a+b) and 10 give a summary of results.

The test product as 0.05 % solution was not active within 5 minutes of exposure time (table 1).

Because of cytotoxicity, in parallel to the end point dilution method the large volume plating method (LVP) was introduced testing the 0.5 % and 0.25 % solution with 5 minutes of exposure time. The mean virus titre was $\log_{10}$ $TCID_{50}$/ml = 6.88 ± 0.32 (table 5) in the first assay and $\log_{10}$ $TCID_{50}$/ml = 6.94 ± 0.38 (table 7) in the second assay.

The test product as 0.5 % solution was active after 5 minutes of exposure time (table 6). Since no residual virus was found in 576 cell culture units at this time point, the result according to the formula of Poisson was ≤ 1.84 $\log_{10}$ $TCID_{50}$. The reduction factor was therefore ≥ 5.04 ± 0.32 (6.88 ± 0.31 $\log_{10}$ $TCID_{50}$ minus ≤ 1.84 $\log_{10}$ $TCID_{50}$). This corresponded to an inactivation of ≥ 99.999 %.

The test product as 0.25 % solution was also active after 5 minutes of exposure time (table 8). Since no residual virus was found in 576 cell culture units at this time point, the result according to the formula of Poisson was ≤ 1.84 $\log_{10}$ $TCID_{50}$. The reduction factor was therefore ≥ 5.10 ± 0.38 (6.94 ± 0.38 $\log_{10}$ $TCID_{50}$ minus ≤ 1.84 $\log_{10}$ $TCID_{50}$). This corresponded to an inactivation of ≥ 99.999 %.

8. Conclusion

The surface disinfectant BARRIER TECH MF Sanitiser Concentrate tested as 0.25 % solution demonstrated activity against bovine coronavirus after an exposure time of 5 minutes under clean conditions. Therefore, the surface disinfectant BARRIER TECH MF Sanitiser Concentrate can be declared as active against bovine coronavirus as follows:

0.25 % **5 minutes** **clean conditions**

Bremen, 07/11/2020

- 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 BARRIER TECH MF Sanitiser Concentrate (0.05 %) tested against bovine coronavirus

Table 2: Raw data for formaldehyde solution (0.7 %) tested against bovine coronavirus

Table 3: Raw data for control of efficacy for suppression of disinfectant's activity (0.5 %)

Table 4: Raw data (bovine coronavirus) for cell sensitivity (0.5 %) (LVP)

Table 5: Determination of virus titre (LVP) (1st assay)

Table 6: Inactivation of bovine coronavirus by BARRIER TECH MF Sanitiser Concentrate (0.5 %) (5 minutes) (LVP)

Table 7: Determination of virus titre (LVP) (2nd assay)

Table 8: Inactivation of bovine coronavirus by BARRIER TECH MF Sanitiser Concentrate (0.25 %) (5 minutes) (LVP)

Table 9 (a+b): Summary of results (end point dilution method) with BARRIER TECH MF Sanitiser Concentrate and bovine coronavirus

Table 10: Summary of results (LVP) with BARRIER TECH MF Sanitiser Concentrate and bovine coronavirus

Legend to the Figures

Figure 1: Virus-inactivating properties of BARRIER TECH MF Sanitiser Concentrate (0.25 %) (LVP)

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

Table 1: Raw data for BARRIER TECH MF Sanitiser Concentrate (0.05 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6920)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	0.05 %	clean conditions	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.a.	4444 4444	4444 4444	4404 4444	0304 0000	0000 0000	n.d.	n.d.
			10	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	0.05 %	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	0003 3200	0000 0000	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 0002	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 formaldehyde solution (0.7 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6920)

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	4434 4403	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	0200 0003	0000 0030	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 control of efficacy for suppression of disinfectant's activity (0.5 %) (#6920)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	clean conditions	n.d.	n.d.	4444 4444	4444 4444	4000 0000	0000 0000	0000 0000	0000 0000	n.d.
corresponding virus control	clean conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 0002	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 (bovine coronavirus) for cell sensitivity (0.5 % solution) (#6920) (LVP)

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	0020 4044	0000 0000	0000 0000	n.d.
test product	1:1,000	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0400 0020	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: Determination of virus titre (LVP) at 20 °C (#6695) (1st assay)

Virus titration	Interfering substance	Exposure time	dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
(beginning of test)	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4040 0000	0000 0000	0000 0000	n.d.
1st control	clean conditions	60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2004 0433	0000 0000	0000 0000	n.d.
2nd control	clean conditions	60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4000 0000	0000 0000	0000 0000	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)

Table 6: Inactivation of bovine coronavirus by BARRIER TECH MF Sanitiser Concentrate (0.5 %) at 20 °C (5 minutes) (LVP, 1:1,000) (#6695)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
clean conditions	plate 1/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 2/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 3/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 4/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 5/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 6/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

t = cytotoxic 0 = no virus detectable 1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Table 7: Determination of virus titre (LVP) at 20 °C (#6920) (2nd assay)

Virus titration	Interfering substance	Exposure time	dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
(beginning of test)	clean conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0003 3200	0000 0000	0000 0000	n.d.
1st control	clean conditions	60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 0002	0000 0000	0000 0000	n.d.
2nd control	clean conditions	60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0000 0334	0000 0000	0000 0000	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)

Table 8: Inactivation of bovine coronavirus by BARRIER TECH MF Sanitiser Concentrate (0.25 %) at 20 °C (5 minutes) (LVP, 1:1,000) (#6920)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
clean conditions	plate 1/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 2/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 3/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 4/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 5/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 6/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

t = cytotoxic 0 = no virus detectable 1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Table 9a: Summary of results (end point dilution method) with BARRIER TECH MF Sanitiser Concentrate and bovine coronavirus

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after …min
				1	5	10	30	60	
test product	0.05 %	clean conditions	2.50	n.d.	6.63±0.41	n.d.	n.d.	n.d.	> 5 (RF = 0.38±0.56)

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

Table 9b: Summary of results (end point dilution method) with BARRIER TECH MF Sanitiser Concentrate 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.	≤ 5.38±0.25	≤ 4.50±0.00	≤ 4.50±0.00	≤ 4.50±0.00	≥ 15 (RF ≥ 2.38±0.41)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	6.88±0.41	n.a.
virus control (+ suppression)	n.a.	clean conditions	n.a.	6.88±0.37	n.d.	n.d.	n.d.	7.00±0.38	n.a.
suppression control	0.5 %	clean conditions	n.d.	n.d.	n.d.	n.d.	5.63±0.25	n.d.	n.a.

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

Table 10: Summary of results (LVP, 1:1,000) with BARRIER TECH MF Sanitiser Concentrate 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	10	30	60	
test product (1)	0.5 %	clean conditions	n.a.	n.d.	≤1.84	n.d.	n.d.	n.d.	5 (RF ≥ 5.04±0.32)
test product (2)	0.25 %	clean conditions	n.a.	n.d.	≤1.84	n.d.	n.d.	n.d.	5 (RF ≥ 5.10±0.38)
virus control (1)	n.a.	clean conditions	n.a.	6.75±0.33	n.d.	n.d.	n.d.	7.13±0.37 6.63±0.25 (Ø6.88±0.32)	n.a.
virus control (2)	n.a.	clean conditions	n.a.	6.88±0.37	n.d.	n.d.	n.d.	7.00±0.38 6.88±0.37 (Ø6.94±0.38)	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.00±0.38	n.a.
sens. product	0.5 % → 1:1,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.33	n.a.

*the number of the brackets gives the number of the corresponding virus control

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

Figure 1: Virus-inactivating properties of BARRIER TECH MF Sanitiser Concentrate (0.25 %) (LVP)

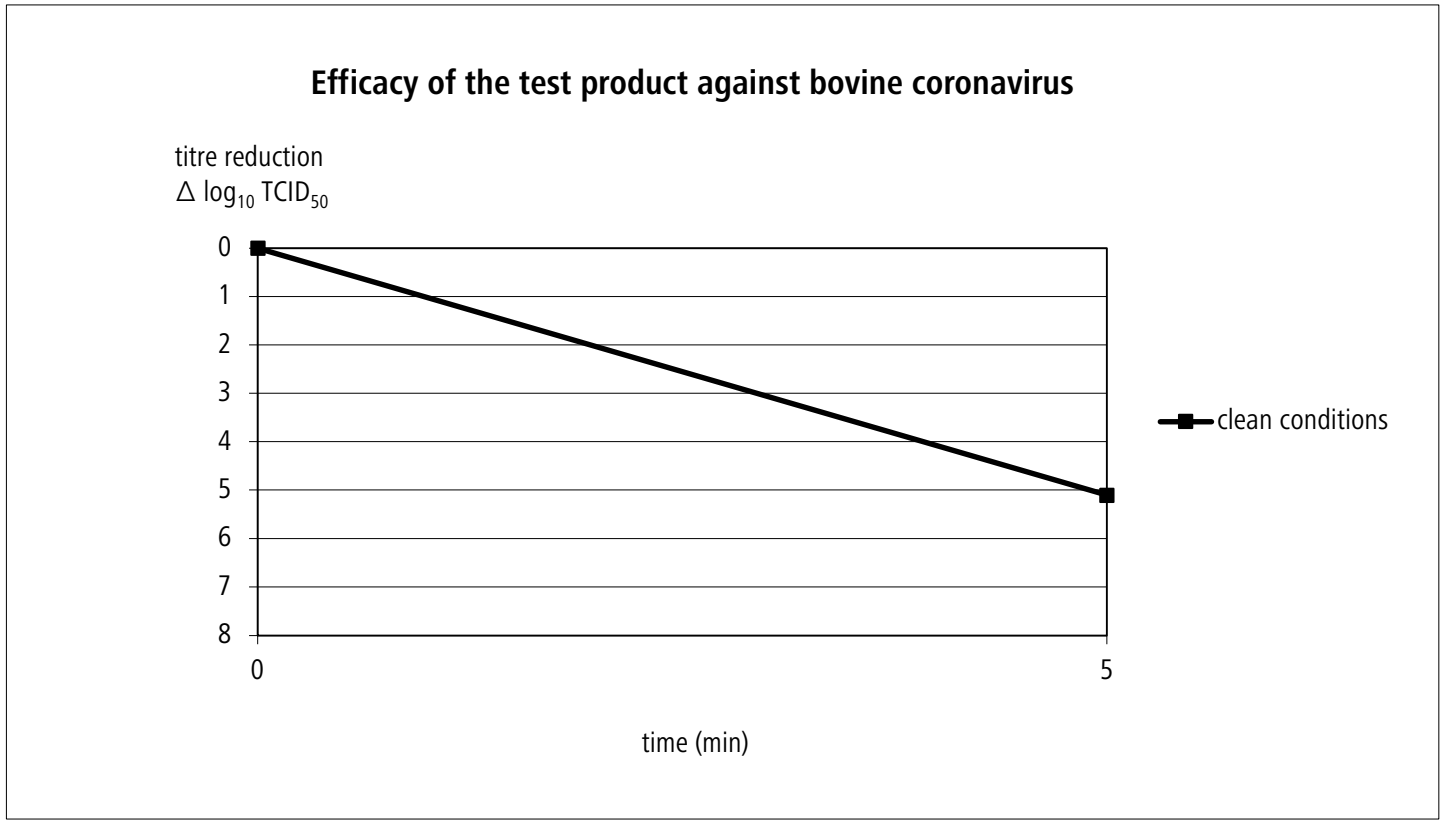

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)