Virus Removal Test Report

Evaluation of the effectiveness on MicronQuick in removing bovine coronavirus (BCoV) from surfaces

To whom it may concern

The objective of the test method was to evaluate the virus removal performance of the Vileda Professional MicronQuick cloth on PVC floor covering plates against bovine coronavirus (BCoV) as surrogate of SARS-CoV-2 coronavirus.

The tests were carried out in May 2020, in the external laboratory Dr. Brill + Partner GmbH, Institute for Hygiene and Microbiology, Norderoog 2, DE - 28259 Bremen.

The test method is based on EN 16615:2015, a quantitative test method for the evaluation of bactericidal and yeasticidal activity on nonporous surfaces with mechanical action employing wipes in the medical area (4-field test).

The test results show that after wiping with the cloth soaked with 40 g purified water,

- no residual virus could be detected on all test fields
- Reduction factor of the two test runs was > 2,88 log (99.86% reduction) on field T1
- Accumulation factor (AF) of the test field T2-T4 is on average <1,50 log TCID50 /ml and in a sum < 1,98 log TCID 50/ml.

Due to the low initial virus titre, it was not possible to reach a 4 log reduction (99.99%) with bovine coronavirus after 10 minutes under clean conditions on field T1 in this quantitative test.

Conclusion

The MicronQuick cloth of Vileda Professional soaked with 40 mL purified water achieved a 99,86 % (log 2.88) reduction of bovine coronavirus (BCoV) with no detectable residual virus.

Date: 17th July 2020

Weinheim, Germany

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TEST REPORT

29/05/2020

Test report L20/0406bBC.1

Evaluation of the effectiveness of

Vileda Professional MicronQuick blue

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

Method: based on EN 16615:2015 (on PVC plates) (clean conditions)

Chemical disinfectants and antiseptics — Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4-field test)

Sponsor:
Freudenberg Home and Cleaning Solutions GmbH
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1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of Vileda Professional MicronQuick blue on PVC plates against bovine coronavirus (BCoV) as surrogate of human coronaviruses using the quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4-field test) based on EN 16615:2015 (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 sample

Manufacturer	Freudenberg Home and Cleaning Solutions GmbH
Name of product	Vileda Professional MicronQuick blue
Confirmation no.	213271
Product diluent recommended by the manufacturer	-
Batch number	-
Application	surface cleaning
Production date	-
Expiry date	-
Composition of floor cleaning cloth	microfiber nonwoven material, prewashed, 40 x 38 cm
Appearance, odour	blue dry wipes product specific
pH-values	-
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	14/04/2020

4. Materials

4.1 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 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)
- Penicillin/ streptomycin (Sigma-Aldrich, article no. P-0781)
- propan-1-ol (Sigma-Aldrich-Chemie GmbH, article no. 33538).

4.2 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 10) 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.

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)
- Analytical balance (Satorius)
- Adjustable and fixed-volume pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, article no. 149026)
- Cell culture flask (Nunc GmbH & Co. KG, article no. 156502)
- Sealed test tubes (Sarstedt AG & Co., article no. 55.468.001)

- Tube, sterile, 15 ml (Sarstedt AG & Co.)
- Petri dishes (Sarstedt AG & Co.)
- Rectangular glass spatula
- FLOQSwaps, sterile, nylon for elution of the residual virus (Copan Diagnostic Inc.)
- Granite block (2.5 kg) 12.1 cm x 8.6 cm x 8.6 cm
- PVC plates with PUR (polyurethane) surface coating 20 x 50 cm (VAH e.V.)

5. Experimental conditions

Test temperature(s)	20 °C ± 2.5 °C
Concentration(s) of test product	not applicable
Appearance of product dilution(s)	not applicable
time(s) after wiping	10 minutes
Interfering substance(s) in the virus inoculum(s)	clean conditions: 0.3 g/l bovine serum albumin (BSA)
Diluent	not applicable
Procedure to stop action	immediate dilution with ice-cold cell medium
Test virus	bovine coronavirus strain L9
Period of analysis	08/05/2019 – 29/05/2020
End of testing	29/05/2020

6. Methods

6.1 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.

6.2 Preparation of test product

Vileda Professional MicronQuick blue were soaked with 40 g VE water per wipe without addition of a disinfectant solution.

6.3 Preparation of the PVC plates

The PVC plates were cleaned with 70.0 % propan-1-ol prior the test. After drying the test fields 1 to 4, each measuring 5 cm x 5 cm, figuring a row at a distance of 5 cm from one another were marked. The row was approximately in the middle of the tested PVC plate (see figure 6.3). The drying controls (DCt0 and DCtx) were performed on an additional test plate – marked with two squares of 5 cm x 5 cm.

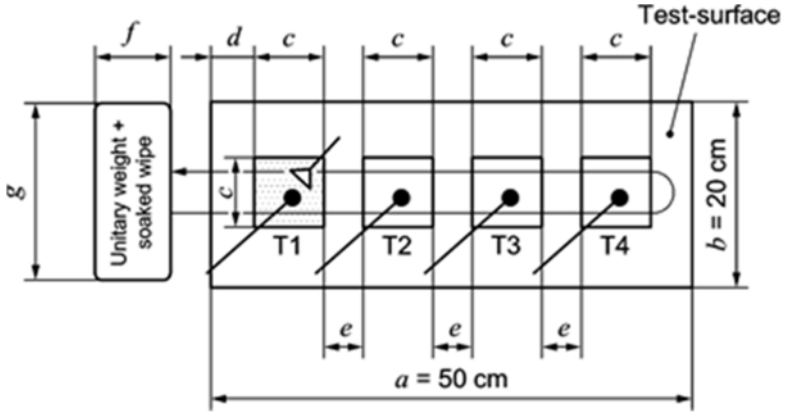

Figure 6.3: Scheme of the markings and the wipe sweep over four test fields on the test-surface. Test field 1 is inoculated with 50 µl of the virus inoculum. The arrow shows the cleaning sweep with the wipe on the granite block. The starting point is in front of test field 1 and the turn is immediately after test field 4. The end point of the wiping process is the starting point after passing the field test 1 for the second time. Schematic representation of the test-surface $a = 50$ cm, $b = 20$ cm; with four areas T1 to T4 (5 cm x 5 cm) and a given range of wiper wipe $c = 5$ cm, $d = 10$ cm, $e = 5$ cm; size of unitary weight (granite block) $f = 8.6$ cm, $g = 12.1$ cm, weight 2.5 kg.

6.4 Preparation of the virus inoculum

Nine volumes of test virus suspension were mixed with one volume of interfering substance solution.

6.5 Inactivation assays

Tests were carried out based on EN 16615 (1) at 20 °C ± 2.5 °C. One plate for the test product plus one for the drying control (DCt0 and DCt10) were prepared.

The plates were marked as described in 6.3 (see Fig. 6.3). Test field 1 or test fields for the drying controls were inoculated with 50 µl of the virus inoculum respectively. The virus inoculum was distributed with a rectangular glass spatula. The rectangular glass spatula was used initially on a blank sample (extra field contaminated with 50 µl virus inoculum) to ensure that field 1 is contaminated with sufficient test suspension. At the latest 60 minutes after drying, the test-surfaces were used for the 4-field test. Vileda Professional MicronQuick blue were soaked with 40 g VE water (200 % dosing per cloth) before introduction to the test. For the wiping process the base of the unitary weight was protected with parafilm, covered with the wipe and fixed with the fingers of the hand, which hold the unitary weight.

The wiping procedure began in front of test field 1, then wiped one second in direction of field 4, turned immediately after test field 4 and wiped back for another second to test field 1 (see Fig. 6.3). After passing the test field 1, the drying time started and the PVC plates left to dry for 10 minutes before starting the elution.

6.6 Elution process

At the end of the drying time, a soaked swap with medium was rubbed over the whole surface of test field 1 and washed out in the tube with 5 ml cell culture medium. The recovery procedure was repeated with the same swab before the swab was put in the tube with the cell culture medium. Afterwards the recovery process on the same field was repeated with a second dry swab till the test field was visually dry. Then, this swab was also put in the same tube with cell culture medium. After the elution process on test field 1 recovery procedure was repeated with the test fields 2, 3 and 4. At the end, the tubes with the swabs and the cell culture medium were vortexed for 30 to 60 seconds and a series of ten-fold dilution of the virus suspension in ice-cold cell culture medium were prepared and were transferred to permissive cells.

6.7 Controls

6.7.1 Virus controls

The recovery procedure from the test fields DCt0 and DCtx was the same as described in 6.6. The recovery from DCt0 started immediately after the drying of the virus inoculum and the recovery from DCtx started immediately after the contact time has elapsed. Additionally, a virus control without drying (VC before) was performed, which is needed for the assessment of the control of efficiency for suppression of disinfectant´s (test product) activity. Therefore, 50 µl of the virus inoculum were mixed with 5 ml cell culture medium an incubated for the duration of drying plus wiping procedure and elution process.

6.7.2 Determination of cytotoxicity

For the determination of cytotoxicity, one test field of the PVC plate was inoculated with 50 µl cell culture medium instead of the virus inoculum. After drying, test was performed as described in 6.5 and 6.6.

6.7.4 Interference control - control of cell susceptibility

For the control of cell susceptibility (in accordance with EN 14476 (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 (test product) and non-pre-treated (PBS) cells as described above. The comparative titration on pre-treated (test product) and non-pre-treated (PBS) cells should show no significant difference (< 1 log_{10}) of virus titre.

6.7.5 Control of efficiency for suppression of disinfectant's (test product) activity

In addition, a control of efficiency for suppression of disinfectant's (test product) activity was included. Therefore, 1,000 µl of the eluate of the cytotoxicity control were mixed with 10 µl of the virus inoculum and incubated for 30 minutes on ice. Finally, a virus titration was performed. The control of efficacy for suppression of disinfectant's (test product) activity should show no decrease (≤ 0.5 log_{10}; EN 14476 (2)) in virus titre compared to the virus control without drying (VC before).

6.7.6 Reference virus inactivation test

As reference for test validation a 0.7 % formaldehyde solution according to EN 14476 (2) was included. 5, 15, 30 and 60 minutes were chosen as contact times. In addition, cytotoxicity of formaldehyde test solution was determined following EN 14476 (2) with dilutions up to 10^{-5}.

6.7.7 Cell culture control

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

6.8 Determination of infectivity

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).

6.9 Calculation and verification of virucidal activity

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

7. Verification of the methodology

In case that the following criteria were fulfilled, examination based on EN 16615 is valid:

a) The titre of the test virus suspension on test field 1 allowed the determination of ≥ 4 log_{10} reduction (table 6). → not valid

b) The difference of the logarithmic titre of the control for suppression of disinfectant's (test product) activity was ≤ 0.5 log_{10} (EN 14476) compared to the virus control before drying (table 7). → valid

c) The comparative titration on pre-treated (test product) and non-pre-treated (PBS) cells showed an acceptable difference (< 1 log_{10}; EN 14476; table 7). → valid

d) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test with formaldehyde was in the range of the values according to EN 14476 (2) (table 7).

Since not all criteria were fulfilled, examination with bovine coronavirus based on EN 16615 is not valid.

8. Results

Results of examination are shown in tables 1 to 7. Tables 1 to 5 show the raw data, table 6 gives the summary of the results for Vileda Professional MicronQuick blue, whereas table 7 shows the summary of results for the controls.

With the test wipes soaked with 40 g VE water per wipe, no residual virus could be detected, but because of the low initial virus titre, it was not able to reach a 4 log reduction with bovine coronavirus after 10 minutes on field 1 under clean conditions in this quantitative 4-field test (table 6). The mean reduction factor of two test runs was ≥ 2.88. The accumulation factor (AF) of the test fields 2 to 4 is on average ≤ 1.50 log_{10} $TCID_{50}$/ml (table 6) and in sum ≤ 1.98 log_{10} $TCID_{50}$/ml (table 6). Here the value for test validation is still under discussion and has to be defined.

9. Conclusion

The surface cleaning wipes Vileda Professional MicronQuick blue were not able to demonstrate effectiveness on field 1 against bovine coronavirus after an exposure time of 10 minutes under clean conditions.

- Dr. Britta Becker -
Head of Laboratory

- Dr. Dajana Paulmann -
Scientific Project Manager

10. 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.

11. 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.

12. Literature

1. Chemical disinfectants and antiseptics – Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4-field test) – Test method and requirements (phase 2/ step2). EN 16615:2015

2. 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)

3. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae.
Brit J Psychol; 2 1908, 227-242.

4. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche.
Arch Exp Path Pharmak; 162, 1931, 480-487.

Appendix

Table 1: Raw data of the virus controls (quantal test; 8 wells) (#6574)

Product	Conc.	Interfering substance	time (min)	Test run	Dilutions (log_{10})								
					1	2	3	4	5	6	7	8	9
virus control before drying (virus inoculum)	n.a.	clean conditions	n.a.	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4000 0003	0000 0000	0000 0000	0000 0000	0000 0000
				n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control after drying (DCt0)	n.a.	clean conditions	0	1	4444 4444	4444 4444	4444 4444	0000 0404	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
				2	4444 4444	4444 4444	4404 0444	0003 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
virus control after drying (DCt10)	n.a.	clean conditions	10	1	4444 4444	4444 4444	4444 0444	0004 0440	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
				2	4444 4444	4444 4444	0400 3044	0000 0000	0000 0000	0000 0000	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 Vileda Professional MicronQuick blue in the 4-field test on PVC plates (quantal test; 8 wells) (#6574)

Product	Conc.	Interfering substance	Drying time after wiping (min)	Test run	Test field	Dilutions (log_{10})								
						0	1	2	3	4	5	6	7	8
Vileda Professional MicronQuick blue	n.a.	clean conditions	10	1	1	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.a.	n.d.	n.d.
					2	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
					3	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
					4	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
Vileda Professional MicronQuick blue	n.a.	clean conditions	10	2	1	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
					2	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
					3	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
					4	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.
product cytotoxicity	n.a.	clean conditions	10	n.a.	1	n.a.	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 for formaldehyde solution (0.7 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#6574)

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	4040 4300	0020 0000	0000 0000	0000 1000	n.d.	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	0000 0000	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	4443 4300	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 4: Raw data of the control of efficacy for suppression of disinfectant's (test product) activity (#6574)

Product	Concentration	Interfering substance	Eluate dilution	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Vileda Professional MicronQuick blue	n.a.	clean conditions	1:10* (= 10^{-0})	n.d.	4444 4444	4444 4444	4444 4444	4404 0004	0300 0300	0000 0000	0000 0000	n.d
virus control	n.a.	clean conditions	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4000 0003	0000 0000	0000 0000	0000 0000	0000 0000

*The undiluted eluate (CT assay, field 1) was mixed with virus inoculum and incubated for 30 min on ice

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 (bovine coronavirus) for cell sensitivity to virus (#6574)

Product	Concentration	Eluate dilution	Dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
Vileda Professional MicronQuick blue	n.a.	undiluted (= 10^{-0})	4444 4444	4444 4444	4444 4444	4444 0330	0320 3000	0000 0000	0000 0000	0000 0000	n.d.
PBS	n.a.	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	0440 0404	0000 0000	0000 0000	0000 0000	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 6: Results with Vileda Professional MicronQuick blue in the 4-field test on PVC plates against bovine coronavirus (#6574)

test run	Drying time after wiping	interfering substance	virus inoculum (log_{10} $TCID_{50}$/ml)	DCto (log_{10} $TCID_{50}$/ml)	DCtx (log_{10} $TCID_{50}$/ml)	field 1 (log_{10} $TCID_{50}$/ml)	RF field 1	field 2 (log_{10} $TCID_{50}$/ml)	field 3 (log_{10} $TCID_{50}$/ml)	field 4 (log_{10} $TCID_{50}$/ml)	Ø AF field 2-4 (log_{10} $TCID_{50}$/ml)	∑field 2 - 4 (log_{10} $TCID_{50}$/ml)
Vileda Professional MicronQuick blue - 1	10 min	clean	5.75	4.75	4.75	≤ 1.50	≥ 3.25	≤ 1.50	≤ 1.50	≤ 1.50	≤ 1.50	1.98
Vileda Professional MicronQuick blue - 2	10 min	clean	5.75	4.38	4.00	≤ 1.50	≥ 2.50	≤ 1.50	≤ 1.50	≤ 1.50	≤ 1.50	1.98
MV			5.75	4.57	4.38	≤ 1.50	≥ 2.88	≤ 1.50	≤ 1.50	≤ 1.50	≤ 1.50	1.98

MV = mean value DC= drying control
AF = accumulation factor RF = reduction factor

Comments: A test product is having virus-inactivating efficacy if a reduction factor of at least ≥ 4 log_{10} (inactivation of ≥ 99.99 %) can be demonstrated on field 1
The accumulation factor demonstrate the residual virus detected on test field 2 - 4

Table 7: Summary of results with the controls

	log_{10} $TCID_{50}$/ml		log_{10} $TCID_{50}$/ml after				reduction (RF)	valid
			5 min	15 min	30 min	60 min		
VCPBS	n.d.	n.d.	n.d.	n.d.	n.d.	7.25	**n.a.**	**n.a.**
1.4 % formaldehyde	n.a.	n.a.	≤5.13	≤4.50	≤4.50	≤4.50	**≥2.75 (15 min)**	**n.a.**
VC (virus inoculum)	5.75	n.d.	n.a.	n.a.	n.a.	n.a.	**n.a.**	**n.a.**
suppression control Vileda Professional MicronQuick blue (undiluted CT eluate)	n.d.	n.d.	n.a.	n.a.	6.25	n.a.	**-0.50**	**Yes**
PBS control	6.00	n.d.	n.a.	n.a.	n.a.	n.a.	**n.a.**	**n.a.**
cell sensitivity control Vileda Professional MicronQuick blue (undiluted CT eluate)	5.63	n.d.	n.a	n.a.	n.a.	n.a.	**0.38**	**Yes**

n.a. = not applicable n.d. = not done DC = drying control AF = accumulation factor
RF = reduction factor

Technical Declaration

Understanding the Dr. Brill + Partner Virus removal reports

Q. Why did you test on Bovine Coronavirus (BCoV) and not SARS-CoV-2 (COVID-19)?

A. There are several reasons:

- EN test methods already exist for removal of Bovine Coronavirus (BCoV) as it causes diarrhoea in calves (20% lethality) making a test protocol economically relevant for cattle breeding

- BCoV does not jump to humans, so it is safe to handle for testing institutes

- Only a limited number of laboratories in the world can currently handle SARS-CoV-2 (COVID-19)

Q. Is it misleading to use tests on Bovine Coronavirus (BCoV) in respect to COVID-19 (SARS-CoV-2)?

A. No, they are directly comparable viruses:

- Bovine coronavirus BCoV belongs biologically to the same subfamily and generic group as SARS-CoV-2

- Bovine coronavirus BCoV is comparable in size, structure and how it is enveloped

Q. You claim 99.86% removal of BCoV virus but none of the tables show this figure

A. In table 6 Results and Section 8 it states the MV (mean value) is a Log 2.88 reduction, mathematically this is 99.86%

Q. The conclusion says the tested product was not able to demonstrate effectiveness on field 1 against bovine coronavirus after an exposure time of 10 minutes under clean conditions.

A. This relates to the test method and not the product tested

The test method didn't allow to achieve log 5 reduction and as such is not considered valid, resulting in the comment "not considered effective" for the product tested.

Q. Why do you state "99.86%" and not 100% if there is "no detectable residual virus"?

A) Test protocols do not allow the statement "100% removed" even if there is no detectable virus left behind (the institute actually measured ZERO residual virus after MicronQuick & MicronSolo wiping).

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Weinheim, Germany