



08/10/2021

Test report L21/01022BC.1

Evaluation of the effectiveness of r-MicroOne Premium CombiSpeed Pro

TEST REPORT

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

Method: Mechanical Surface wiping procedure – quantitative test method for the evaluation of virus removal on non-porous surfaces (4-field test) (on PVC plates) (clean conditions)

Sponsor:

Freudenberg Home and Cleaning Solutions GmbH
Regional Technical Centre Europe
Vileda Professional Science & Training Center
Hoehnerweg 2-4
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1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of r-MicroOne Premium CombiSpeed Pro on PVC plates against bovine coronavirus (BCoV) as surrogate of human coronaviruses using a Mechanical Surface wiping procedure – quantitative test method for the evaluation of virus removal on non-porous surfaces 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	r-MicroOne Premium CombiSpeed Pro
Additional name(s)	r-MicroOne Premium SWEP Duo; r-MicronOne Premium UltraSpeed Pro
Internal product identifier	21/00921-001
Confirmation no.	225459
Product diluent recommended by the manufacturer	-
Batch number	-
Application	surface cleaning
Production date	-
Expiry date	-
Composition of cleaning cloth	100% polyester
Appearance, odour	white single use mop product specific
pH-values	-
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	12/07/2021

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 (Sigma, article no. BCCC6626)
- 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 (Gibco, 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 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.

4.3 Apparatus, glassware and small items of equipment

- CO₂ 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	29/09/2021 – 05/10/2021
End of testing	08/10/2021

6. Methods

6.1 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.

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6.2 Preparation of test product

r-MicroOne Premium CombiSpeed Pro were soaked with 26 ml VE water per mop 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.

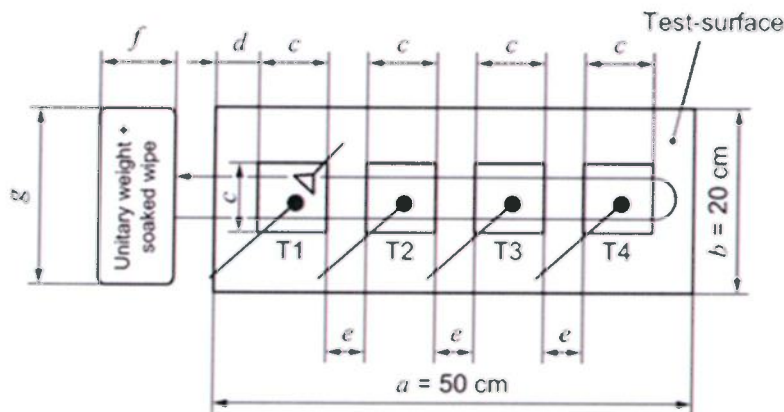


Figure 6.3: Scheme of the markings and the wipe sweep over four test fields on the test-surface. Test field 1 is inoculated with 50 μ l of the virus inoculum. The arrow shows the cleaning sweep with the wipe on the granite block. The starting point is in front of test field 1 and the turn is immediately 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 as described in EN 16615 (1) at $20 \text{ }^\circ\text{C} \pm 2.5 \text{ }^\circ\text{C}$. 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. r-MicroOne Premium CombiSpeed Pro were soaked with 26 ml VE water before introduction to the test. For the wiping process the base of the unitary weight was protected with parafilm, covered with the mop 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 swab 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 and 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.

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6.7.3 Interference control - control of cell susceptibility

For the control of cell susceptibility 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.

6.7.4 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 ($\leq 0.5 \log_{10}$) in virus titre compared to the virus control without drying (VC before).

6.7.5 Reference virus inactivation test

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

6.7.6 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 CO₂-atmosphere (5.0 % CO₂ - content). Finally, cultures were observed for cytopathic effects for six days of inoculation. The infectious dose (TCID₅₀) was calculated according to the method of Spearman (2) and Kärber (3).

In parallel to the end point dilution method the large volume plating method (LVP) was introduced. Following the large volume plating method (EN 14476 (4), section 5.5.4.3) the eluates of the test field 1 were further diluted 1:10 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 log₁₀ reduction of virus titre. Calculation of virus titre follows formula of Taylor or Poisson (EN 14476 (4), section B.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. Results

Results of examination are shown in tables 1 to 9. Tables 1 to 6 show the raw data, tables 7 and 8 give the summary of the results for r-MicroOne Premium CombiSpeed Pro, whereas table 9 shows the summary of results for the controls.

With the test mops soaked with 26 ml VE water per mop, a small amount of residual virus could be detected with the endpoint dilution method after 10 minutes on field 1 under clean conditions in this quantitative 4-field test (table 7). The mean reduction factor of two test runs was ≥ 3.72 . The accumulation factor (AF) of the test fields 2 to 4 is on average $\leq 1.50 \log_{10} \text{TCID}_{50}/\text{ml}$ (table 6) and in sum $\leq 1.98 \log_{10} \text{TCID}_{50}/\text{ml}$ (table 7).

In parallel to the end point dilution method the large volume plating method (LVP) was introduced testing the eluates of test field 1. The eluates were further diluted 1:10.

Testing the 1:10 dilution of the eluates of test field 1, residual virus was found in 44 and 43 of 288 cell culture units in both test runs (table 3 and 8). The mean reduction factor of two test runs was 4.02.

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8. Conclusion

After the cleaning procedure with the tested cleaning mop on the surface of the PVC-plate, a virus removal of 99.99 % could be achieved for r-MicroOne Premium CombiSpeed Pro.

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9. 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
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3. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche.
Arch Exp Path Pharmac; 162, 1931, 480-487.
4. 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)

Appendix

- Table 1: Raw data of the virus controls
- Table 2: Raw data of r-MicroOne Premium CombiSpeed Pro in the 4-field test on PVC plates (end point dilution method)
- Table 3: Raw data of r-MicroOne Premium CombiSpeed Pro in the 4-field test on PVC plates (LVP method)
- Table 4: Raw data for formaldehyde solution (0.7 %) against bovine coronavirus
- Table 5: Raw data of the control of efficacy for suppression of disinfectant's (test product) activity
- Table 6: Raw data (bovine coronavirus) for cell sensitivity to virus
- Table 7: Results with r-MicroOne Premium CombiSpeed Pro in the 4-field test on PVC plates against bovine coronavirus (end point dilution)
- Table 8: Results with r-MicroOne Premium CombiSpeed Pro in the 4-field test on PVC plates against bovine coronavirus (LVP)
- Table 9: Summary of results with the controls



Table 1: Raw data of the virus controls (quantal test; 8 wells) (#7592)

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.	n.d.	n.d.	4444	4444	4444	0304	0000	0000	0000	n.d.
				n.a.	n.d.	n.d.	4444	3444	2440	0000	0000	0000	n.d.	n.d.
virus control after drying (DcT0)	n.a.	clean conditions	0	1	n.d.	4444	4444	4444	3444	0003	0000	0000	n.d.	n.d.
				2	n.d.	4444	4444	4400	4020	0000	0000	0000	n.d.	n.d.
virus control after drying (DcT10)	n.a.	clean conditions	10	1	n.d.	4444	4444	4444	4003	0000	0000	0000	n.d.	n.d.
				2	n.d.	4444	4444	4444	4444	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)

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Table 2: Raw data of r-MicroOne Premium CombiSpeed Pro in the 4-field test on PVC plates (quantal test; 8 wells) (end point dilution method) (#7592)

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								
r-MicroOne Premium CombiSpeed Pro	n.a.	clean conditions	10	1	1	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.				
					2	tttt	0004	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.	
					3	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
					4	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
r-MicroOne Premium CombiSpeed Pro	n.a.	clean conditions	10	2	1	tttt	4000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.			
					2	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.	
					3	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
					4	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
product cytotoxicity	n.a.	clean conditions	10	n.a.	1	tttt	0000	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.	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)

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Table 3: Raw data of r-MicroOne Premium CombiSpeed Pro in the 4-field test on PVC plates (LVP of test field 1) (#7592)

Product	Test run	Row	1	2	3	4	5	6	7	8	9	10	11	12		
r-MicroOne Premium CombiSpeed Pro	1	plate 1/3	0004 4000	0000 0000	0000 4040	0000 0000	0000 0040	0000 0040	0000 0004	0000 0004	0400 0004	0004 0000	0000 4000	0004 0000		
		plate 2/3	0400 0000	0400 0040	0000 0040	0000 0400	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0004 0000	0040 4400	4000 4040	4000 4000	
		plate 3/3	0000 0000	0400 0000	0040 0000	0400 0000	0000 4000	0000 0000	0000 4004	0000 0000	0000 0000	0000 0400	0040 4040	0004 0400	0000 0000	0040 0040
		plate 1/3	0000 0400	0040 0004	0000 0040	0000 0400	0000 0000	0000 0000	0000 0000	0000 0400	0000 0400	0000 0004	0000 0000	0000 0400	0000 0400	0000 0404
		plate 2/3	0000 0440	0000 0040	0000 4040	0000 0000	0000 0000	0040 0000	4004 0000	0000 0400	0000 0000	0044 0000	0040 0400	0440 0000	0004 0440	4000 0044
		plate 3/3	0000 0000	0000 0000	0000 4400	0000 0000	0000 0000	0000 0000	0000 0000	0040 0000	0000 0000	0004 0000	0000 0000	0000 0000	0000 0040	4004 0000

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Table 4: Raw data for formaldehyde solution (0.7 %) tested against bovine coronavirus at 20 °C (quantal test; 8 wells) (#7592)

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	n.d.	tttt	tttt	0343	0000	0000	0000	0000	0000	n.d.	n.d.	
			15	n.d.	tttt	tttt	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			30	n.d.	tttt	tttt	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	60	n.d.	tttt	tttt	0000	0000	0000	0000	0000	0000	n.d.	n.d.	
			n.a.	n.d.	tttt	tttt	0000	0000	0000	0000	0000	0000	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.	n.d.	n.d.	
			60	n.d.	n.d.	4444	4444	4444	0030	0000	0000	0000	0000	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)

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Table 5: Raw data of the control of efficacy for suppression of disinfectant's (test product) activity (#7592)

Product	Concentration	Interfering substance	Eluate dilution	Dilutions (log ₁₀)										
				1	2	3	4	5	6	7	8	9		
r-MicroOne Premium CombiSpeed Pro	n.a.	clean conditions	1:10* (= 10 ⁻⁶)	n.d.	4444	4444	4444	4444	0040	0000	0000	0000	0000	n.d.
virus control	n.a.	clean conditions	n.a.	n.d.	n.d.	4444	4444	4444	0304	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)

t = cytotoxic

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

Table 6: Raw data (bovine coronavirus) for cell sensitivity to virus (#7592)

Product	Concentration	Eluate dilution	Dilutions (log ₁₀)											
			1	2	3	4	5	6	7	8	9			
r-MicroOne Premium CombiSpeed Pro	n.a.	1:10 (= 10 ⁻¹)	n.d.	n.d.	4444	4444	4444	0000	0000	0000	0000	0000	0000	n.d.
PBS	n.a.	n.a.	n.d.	n.d.	4444	3444	4434	0300	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)

t = cytotoxic

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Table 7: Results with r-MicroOne Premium CombiSpeed Pro in the 4-field test on PVC plates against bovine coronavirus (end point dilution) (#7592)

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)	Σ Field 2 - 4 (log ₁₀ TCID ₅₀ /ml)
1	10 min	clean	6.13	5.63	5.25	≤ 1.63	≥ 3.63	≤ 1.50	≤ 1.50	≤ 1.50	≤ 1.50	1.98
2	10 min	clean	n.d.	5.13	5.50	≤ 1.63	≥ 3.88	≤ 1.50	≤ 1.50	≤ 1.50	≤ 1.50	1.98
	MW		6.13	5.38	5.38	≤ 1.63	≥ 3.75	≤ 1.50	≤ 1.50	≤ 1.50	≤ 1.50	1.98

MW = mean value DC= drying control

AF = accumulation factor RF = reduction factor

Comments: The accumulation factor demonstrates the residual virus detected on test field 2 - 4

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Table 8: Results with r-MicroOne Premium CombiSpeed Pro in the 4-field test on PVC plates against bovine coronavirus (LVP) (#7592)

test run	Drying time after wiping	interfering substance	virus inoculum (log ₁₀ TCID ₅₀ /ml)	D _{Cto} (log ₁₀ TCID ₅₀ /ml)	D _{Ctx} (log ₁₀ TCID ₅₀ /ml)	field 1 (log ₁₀ TCID ₅₀ /ml)	RF field 1
1	10 min	clean	6.13	5.63	5.25	1.36	3.89
2	10 min	clean	n.d.	5.13	5.50	1.35	4.15
MW							
5.38							

MW = mean value

DC= drying control

AF = accumulation factor

RF = reduction factor

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Table 9: 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.	7.00	n.a.	
1.4 % formaldehyde	n.a.	n.a.	≤4.50	≤4.50	≤4.50	≤4.50	≥2.50 (15 min)	
VC (virus inoculum)	6.13	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	
suppression control r-MicroOne Premium CombiSpeed Pro (undiluted CT eluate)	n.d.	n.d.	n.a.	n.a.	5.75	n.a.	Yes	
PBS control cell sensitivity control	5.75	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	
r-MicroOne Premium CombiSpeed Pro (CT eluate 1:10)	5.88	n.d.	n.a.	n.a.	n.a.	-0.13	Yes	

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

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