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TEST REPORT

28.08.2012

Test report no. F12ML1412-1B

Evaluation of the effectiveness of

ProCare+Handcare

Testvirus:	Bovine Viral Diarrhea Virus (BVDV) (Surrogate of HCV)
Method:	according to the guideline of DVV and RKI (dating 01.08.2008)

Sponsor:
Hygicare ApS
Vesterbrogade 76
DK-1620 Cph. V
Denmark

Norderoog 2, D-28259 Bremen
Tel.: +49 (0) 421-27819102, Fax: +49 (0) 421-2760283
MikroLab.GmbH@t-online.de, http://www.mikrolab-gmbh.de

1. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

2. Identification of sample

Name of product	ProCare+Handcare
Manufacturer	Hygicare
Application	hand hygiene
Lot no.	H32061
Expiry date	June 15th 2013
Date of production	-
Substance(s) and concentration(s) in 100 g	ethanol (CAS no.: 64-17-5) phenoxyethanol 122-99-6 (CAS no.: 122-99-6) didecyldimethylammonium chloride (CAS no.: 7173-51-5)
Appearance and odour	clear, colourless gel; product specific
pH-value (s) (in hard water)	undiluted: 7.11 (20°C)
Conditions of storage	room temperature in the dark (area with limited access)
Date of receipt at laboratory	29.05.2012

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Lonza Group Ltd., catalogue no. BE12-125F)
- Fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % Formaldehyde solution (Chemisch-technologisches Laboratorium Dr. Melzer, D-28199 Bremen)
- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)

3.2 Virus and cells

BVDV strain NADL (VR-534) was obtained from Dr. Stephanie Bendtfeld, Institute of Virology at the School of Veterinary Medicine Hannover (Tierärztliche Hochschule, D-30559 Hannover). Prior to inactivation assays, the virus was passaged once in *primary bovine kidney cell*s and five times in *KOP-R cells* (primary cells from bovine oropharyngeal tissue). *KOP-R cells* originated from the Friedrich-Löffler-Institut, Bundesforschungsinstitut für Tiergesundheit (formerly Bundesforschungsanstalt für Viruskrankheiten der Tiere, isle of Riems) (Dr. R. Riebe, catalogue no. RIE 244). In the inactivation assays *ekl cells* (embryonal cells from bovine lung tissue) were used. These cells originated from Mrs. A. Kyas (Henkel KGaA, D-40191 Düsseldorf).

3.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- 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)
- Transferpettor® (Brand GmbH & Co. KG, Wertheim, Germany)
- Polysterol 96-well microtitre plates (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Cell culture flasks (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht, Germany)
- MicroSpin^{TM} S-400 HR columns (GE Healthcare, D-79021 Freiburg, Germany).

4. Experimental conditions

Test temperature	20 °C ± 0.5 °C
Concentration of test product	50.0 %, 10.0 % and 0.1 % (non-active range) solutions
Contact times	30 and 60 seconds
Interfering substance	fetal calf serum (FCS)
Procedure to stop action of disinfectant	immediate dilution, gel filtration and assay following the method of Lycke
Diluent	water of standardised hardness
Virus strain	BVDV strain NADL
Date of testing	29.05.2012 – 28.08.2012
End of testing	28.08.2012

5. Methods

5.1 Preparation of test virus suspension

For the preparation of the test virus suspension, *KOP-R cells*, which were cultivated with Eagle's Minimum Essential Medium (EMEM) supplemented with L-glutamine, sodium pyruvate and 10 % or 2 % fetal calf serum (FCS), were infected with BVDV (stock virus suspension). As soon as cells showed a constant cytopathic effect, they were subjected to a rapid freeze/thawing procedure. This was followed by low-speed centrifugation (10 min and 1000 x g) in order to sediment cell debris. After aliquotation, test virus suspension was stored at –80 °C.

5.2 Preparation of disinfectant (dilutions)

The test product was evaluated as 50.0 %, 10.0 % and 0.1 % solutions. Due to the addition of test virus suspension and interfering substance these concentrations were multiplied by a factor of 1.25.

5.3 Inactivation assays and controls

Tests were carried out in accordance with the DVV and RKI guideline (1). Eight parts by volume of the disinfectant were mixed with one part by volume of test virus suspension and one part by volume of Aqua bidest. In tests with interfering substance, instead of Aqua

bidest., one part by volume of fetal calf serum was added. Immediately at the end of the chosen exposure time, activity of the disinfectant was stopped by serial dilutions.

Due to a more convenient handling and due to a limited amount of test virus suspension, the volumes in the inactivation assay were 0.1 ml test virus suspension, 0.1 ml interfering substance (FCS) and 0.8 ml test product.

Virus controls were incorporated after the longest exposure time. One part by volume of test virus suspension was mixed with nine parts by volume of Aqua bidest. or with one part by volume of FCS and eight parts by volume of Aqua bidest.

A control was carried out with one part by volume of test virus suspension, four parts by volume of PBS (0.1 M, pH value 7.0) and five parts by volume of 1.4 % formaldehyde solution. 5, 15 and 30 minutes were chosen as contact times.

For determination of cytotoxicity of the disinfectant, two parts by volume of Aqua bidest. were mixed with eight parts by volume of the disinfectant, diluted with ice-cold EMEM and inoculated onto permissive cells. Values are given as $\log_{10}CD_{50}$/ml (in analogy to $\log_{10}TCID_{50}$/ml).

Since the cytotoxicity did not allow following the reduction of residual infectivity titer over the range of four $\log_{10}$ steps, ready to use MicroSpin™ S-400 HR columns (GE Healthcare, D-79021 Freiburg, Germany) were used in order to remove the cytotoxic agents according to instructions of the manufacturer. Virus controls without columns were included.

Inactivation tests were carried out in sealed test tubes in a water bath at 20 °C ± 0.5 °C. Aliquots were retained after appropriate exposure times, and the residual infectivity was determined.
The inactivation experiments were run in two independent assays (two different days).

A control of efficiency for suppression of disinfectant activity was not included since at the end of the exposure time dilutions were done immediately.

Furthermore, a cell control was incorporated.

5.4 Determination of infectivity

Infectivity was determined by means of end point dilution titration in a micro-procedure. For this, samples were diluted with ice-cold EMEM and 100 µl of each dilution were placed in 8 wells of a sterile polystyrene flat bottomed microtitre plate. 100 µl of *ekl cells* were added into the plates one day earlier. Suspension was adjusted to reach approximately 10-15 x 10^3 cells per well. Incubation was at 37 °C in a CO_2-atmosphere (5.0 % CO_2 - content). Finally, cultures were observed for cytopathic effects for ten days of inoculation. The infective dose ($TCID_{50}$) (with 95 % level of confidence) was calculated according to the method of Spearman (2) and Kärber (3) with the following formula:

$$- \log_{10} TCID_{50} = X_0 + 0.5 - \sum r/n$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

r = number of positive determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

5.5 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 (virus control). The difference is given as reduction factor (RF).

According to the guideline (Leitlinie) of DVV/RKI, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if within the recommended exposure period the titre is reduced at least by four $\log_{10}$ steps.

5.6 Inactivation assay following the method of Lycke

Following a modified procedure as described by Lycke (4), the test mixture was further diluted 1:2,000 in EMEM and then 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. This method is necessary for those products which demonstrate a great cytotoxicity.

100 µl of the test virus suspension and 100 µl Aqua bidest. and FCS, respectively were mixed with 800 µl disinfectant at 20°C. At the end of the exposure time (30 seconds) 31.25 µl

of the mixture were immediately added to 62.5 ml EMEM (1:2,000 dilutions) and then the volume was distributed in six microtitre plates (108 µl / well). After 10 days of inoculation cultures were observed for cytopathic effects. The calculation of virus titre followed the formula of Lycke:

$$-\log_{10} = [1.4 \times \ln (1-p)].$$

p is meaning the relation between the positive wells with virus detection in comparison to the total number of wells.

For the control of cell sensitivity 200 µl Aqua bidest. or 100 µl Aqua bidest. and 100 µl FCS, respectively, were mixed with 800 µl disinfectant (PBS as control). Then, an aliquot was diluted 1:2,000 and 108 µl of this dilution were added to the wells of the microtitre plates with a preformed monolayer of *ekl 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.

Determination of the initial virus titre was performed in a quantitative suspension tests by a fivefold assay (see 5.3). The virus-inactivating properties of the test product were calculated by subtracting the virus titre in the test mixture from the virus control.

6. Results

6.1 Determination of cytotoxicity

In parallel with the inactivation tests, the cytotoxicity of ProCare+HandCare (50.0 %, 10.0 % and 0.1 %) and 0.7 % formaldehyde was measured.

The formaldehyde solution was toxic for the *ekl cells* in the 1:1,000 dilutions. This corresponded to a $\log_{10}CD_{50}$/ml of 4.50 (Table 1).

Examinations also showed that ProCare+HandCare achieved a $\log_{10}CD_{50}$/ml of 4.50 (50.0 %) and 3.50 (10.0 %), respectively (Table 1). After introduction of the columns, the cytotoxicity was reduced to 1.50 to 2.50 (10.0 % and 50.0 %).

These tests to measure cytotoxicity are imperative, because in this manner the lower detection threshold for non-inactivated BVDV could be determined.

6.2 Virus-inactivating properties of formaldehyde control

Formaldehyde (0.7 %) reduced the BVDV titre after five and 15 minutes by ≥ 0.88 ± 0.35 and ≥ 0.88 ± 0.35 $\log_{10}$ steps. After 30 and 60 minutes a reduction factor of ≥ 0.88 ± 0.35 was measured (Table 5).

6.3 Virus-inactivating properties of disinfectant

Results of inactivation assays are demonstrated in tables 2 to 8 (raw data s. appendix). ProCare+HandCare was examined as 50.0 %, 10.0 % and 0.1 % solutions. 30 and 60 seconds were chosen as exposure times in these experiments.

ProCare+HandCare (50.0 %) was active against BVDV after 30 seconds of exposure time. The reduction factors were ≥ 0.88 ± 0.35 (assay without soil load) and ≥ 1.13 ± 0.18 (assay with FCS) (table 2). After introduction of the columns the reduction factors were ≥ 2.75 ± 0.23 (assay without soil load) and ≥ 3.00 ± 0.25 (assay with FCS) (table 3). Due to the cytotoxicity a reduction of 4 $\log_{10}$ steps could not be demonstrated.

Tested as 10.0 % solution, ProCare+HandCare was also active against BVDV after 30 seconds of exposure time. The reduction factors were ≥ 1.88 ± 0.35 and ≥ 1.88 ± 0.35 (assays without soil load) (mean ≥ 1.88 ± 0.25) and ≥ 2.13 ± 0.25 and ≥ 2.00 ± 0.39 (assays with FCS) (mean ≥ 2.07 ± 0.23) (tables 4 and 5). After introduction of the columns the reduction factors were ≥ 3.50 ± 0.27 (assay without soil load) and ≥ 4.00 ± 0.25 (assay with FCS) (tables 6 and 7). Due to the cytotoxicity a reduction of 4 $\log_{10}$ steps could not be demonstrated in all assays.

Additionally, the product was examined as 0.1 % solution in the presence of FCS for demonstrating the non-active range. The reduction factor after 60 seconds was 0.00 ± 0.71 (table 8).

6.4 Virus-inactivating properties of the disinfectant following the method of Lycke*

As mentioned above, a 4 $\log_{10}$ reduction could not be measured in the quantitative suspension test due to high cytotoxicity. Therefore, the method of Lycke was introduced.
A control for cell sensitivity according to Lycke was evaluated by a comparative titration. The comparative virus titration on cells treated with the disinfectant (1.0 %) (5.63 ± 0.49 $\log_{10}$TCID$_{50}$/ml) and PBS (5.50 ± 0.46 $\log_{10}$TCID$_{50}$/ml) resulted in a difference of ≤ 0.5 $\log_{10}$ of virus titre in the presence of the disinfectant demonstrating that virus replication was not inhibited (Appendix table 9).

The results are given in Appendix tables 9 to 11. The virus titres in the fivefold assay were $\log_{10}$ TCID$_{50}$ / ml = 5.51 (assay without soil load) and 5.70 (assay with FCS).

* Method not accredited

These values corresponded to the virus amount in the assays to 4.00 (assay without soil load) and 4.19 (assay with FCS) $\log_{10}$ $TCID_{50}$.
Since in 576 cell culture units in the assays without soil load no residual virus could be detected the result according to the formula of Lycke is $\log_{10} = 0$. The reduction factor is therefore $\log_{10}$ 4.00 minus $\log_{10}$ 0 = 4.00 after an exposure time of 30 seconds.
In the presence of FCS the result is $\log_{10} = 0$. The reduction factor is therefore $\log_{10}$ 4.19 minus $\log_{10}$ 0 = 4.19 after an exposure time of 30 seconds.

In summary, ProCare+HandCare was able to inactivate BVDV (surrogate of Hepatitis C Virus) as follows:

10.0 % 30 seconds

- Dr. J. Steinmann -
Wiss. Techn. Leiter der MikroLab GmbH

7. 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.

8. Records to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between MikroLab GmbH and the sponsor will be stored in the archives at MikroLab GmbH.

9. Literature

1. Leitlinie der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) e.V. und des Robert Koch-Institutes (RKI) zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren in der Humanmedizin (in der Fassung vom 1. August 2008)
Bundesgesundheitsbl., 51, 2008, 936-445

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

4. Lycke, E.: Studies of the Inactivation of Poliomyelitis Virus by Formaldehyde.
Arch Ges Virusforsch; 7, 1957: 483-493

Table 1: Cytotoxicity of ProCare+HandCare and 0.7 % formaldehyde before and after the treatment with the MicroSpin™ S-400 HR columns

before treatment	conc.	soil load	dilutions 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
product	50.0%	Aqua bidest.	t	t	t	-	-
product	50.0%	10.0% FCS	t	t	t	-	-
product	10.0%	Aqua bidest.	t	t	-	-	-
product	10.0%	10.0% FCS	t	t	-	-	-
product	0.1%	Aqua bidest.	n.d.	n.d.	n.d.	n.d.	n.d.
product	0.1%	10.0% FCS	t	-	-	-	-
formaldehyde	0.7%	PBS	t	t	t	-	-
after treatment	**conc.**	**soil load**	**dilutions 10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
product	50.0%	Aqua bidest.	t	-	-	-	-
product	50.0%	10.0% FCS	t	-	-	-	-
product	10.0%	Aqua bidest.	-	-	-	-	-
product	10.0%	Aqua bidest.	-	-	-	-	-

t = cytotoxic n.d. = not done

Table 2: Inactivation of BVDV by ProCare+HandCare (50.0 %) and formaldehyde (0.7 %) in a quantitative suspension test at 20 °C

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	240 s	30 s	60 s	120 s	240 s	
test product	50.0%	Aqua bid.	≤4.50±0.00	≤4.50±0.00	n.d.	n.d.	≥0.88±0.35	≥0.88±0.35	n.a.	n.a.	≥ 30 s
test product	50.0%	10.0% FCS	≤4.50±0.00	≤4.50±0.00	n.d.	n.d.	≥1.13±0.18	≥1.13±0.18	n.a.	n.a.	≥ 30 s
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	5.38±0.49	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	5.63±0.25	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

Table 3: Inactivation of BVDV by ProCare+HandCare (50.0 %) and formaldehyde (0.7 %) in a quantitative suspension test at 20 °C (with columns)

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	240 s	30 s	60 s	120 s	240 s	
test product	50.0%	Aqua bid.	≤2.50±0.00	n.d.	n.d.	n.d.	≥2.75±0.23	n.a.	n.a.	n.a.	≥ 30 s
test product	50.0%	10.0% FCS	≤2.50±0.00	n.d.	n.d.	n.d.	≥3.00±0.25	n.a.	n.a.	n.a.	≥ 30 s
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	5.25±0.33	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	5.50±0.35	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

Table 4: Inactivation of BVDV by ProCare+HandCare (10.0 %) and formaldehyde (0.7 %) in a quantitative suspension test at 20 °C (1st assay)

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	240 s	30 s	60 s	120 s	240 s	
test product	10.0%	Aqua bid.	≤3.50±0.00	≤3.50±0.00	n.d.	n.d.	≥1.88±0.35	≥1.88±0.35	n.a.	n.a.	≥ 30 s
test product	10.0%	10.0% FCS	≤3.50±0.00	≤3.50±0.00	n.d.	n.d.	≥2.13±0.18	≥2.13±0.18	n.a.	n.a.	≥ 30 s
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	5.38±0.49	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	5.63±0.25	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

Table 5: Inactivation of BVDV by ProCare+HandCare (10.0 %) and formaldehyde (0.7 %) in a quantitative suspension test at 20 °C (2nd assay)

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	240 s	30 s	60 s	120 s	240 s	
test product	10.0%	Aqua bid.	≤3.50±0.00	n.d.	n.d.	n.d.	≥1.88±0.35	n.a.	n.a.	n.a.	≥ 30 s
test product	10.0%	10.0% FCS	≤3.50±0.00	n.d.	n.d.	n.d.	≥2.00±0.39	n.a.	n.a.	n.a.	≥ 30 s
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	≤4.50±0.00	≤4.50±0.00	≤4.50±0.00	≤4.50±0.00	≥0.88±0.35	≥0.88±0.35	≥0.88±0.35	≥0.88±0.35	≥ 5 min
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	5.38±0.49	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	5.50±0.55	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

Table 6: Inactivation of BVDV by ProCare+HandCare (10.0 %) and formaldehyde (0.7 %) in a quantitative suspension test at 20 °C (with columns) (1st assay)

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	240 s	30 s	60 s	120 s	240 s	
test product	10.0%	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
test product	10.0%	10.0% FCS	≤1.50±0.00	n.d.	n.d.	n.d.	≥4.00±0.25	n.a.	n.a.	n.a.	30 s
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	5.50±0.35	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

Table 7: Inactivation of BVDV by ProCare+HandCare (10.0 %) and formaldehyde (0.7 %) in a quantitative suspension test at 20 °C (with columns) (2nd assay)

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	240 s	30 s	60 s	120 s	240 s	
test product	10.0%	Aqua bid.	≤1.50±0.00	n.d.	n.d.	n.d.	≥3.50±0.27	n.a.	n.a.	n.a.	≥ 30 s
test product	10.0%	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	5.00±0.38	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

Table 8: Inactivation of BVDV by ProCare+HandCare (0.1 %) and formaldehyde (0.7 %) in a quantitative suspension test at 20 °C

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	240 s	30 s	60 s	120 s	240 s	
test product	0.1%	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
test product	0.1%	10.0% FCS	5.38±0.41	5.75±0.44	n.d.	n.d.	0.13±0.69	0.00±0.71	n.a.	n.a.	> 30 s
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/ml with 95% level of confidence after				Reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			5 min	15 min	30 min	60 min	5 min	15 min	30 min	60 min	
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	5.50±0.55	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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