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28.08.2012

Test report no. P12ML1412-1V

Evaluation of the effectiveness of

ProCare+Handcare

Test virus: vaccinia virus strain Elstree

Method: according to the guideline of DVV and RKI (dating 01.08.2008)

TEST REPORT

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 ApS
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-Bremen)
- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)

3.2 Virus and cells

Vaccinia virus strain Elstree originated from the Institute of Medical Virology and Immunology of the University of Essen, D-45122 Essen. Before inactivation assays, virus had been passaged 10 times in *GMK AH-1 cells* (green monkey kidney cell line), three times in *HeLa cells* and five times in *Vero cells* (monkey kidney cell line).

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, 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	undiluted (90.0 %) and as 10.0 % and 1.0 % (non-active range) solutions
Contact times	30, 60 and 120 seconds
Interfering substance	fetal calf serum (FCS)
Procedure to stop action of disinfectant	immediate dilution and gel filtration
Diluent	water of standardised hardness (10.0 % and 1.0 % solutions)
Virus strain	vaccinia virus strain Elstree
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 preparation of test virus suspension, *Vero cells* (ATCC CC81; permanent monkey kidney cells) were cultivated with Eagle`s Minimum Essential Medium and 10 % or 2 % fetal calf serum.

Vero cells were infected with a multiplicity of infection of 0.1. After cells showed a cytopathic effect, they were subjected to a twofold freeze/thaw procedure followed by a 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 undiluted. Due to the addition of test virus suspension and interfering substance a 90.0 % solution resulted (0.1 part test virus suspension + 0.9 parts interfering substance + 9 parts disinfectant). The product was also tested as 10.0 % and 1.0 % (1 part test virus suspension + 1 parts interfering substance + 8 parts disinfectant) (demonstration of non-active range) solutions.

The 10.0 % and 1.0 % solutions were prepared with water of standardised hardness immediately before the inactivation tests.

5.3 Inactivation assays and controls

Tests were carried out in accordance with the DVV and RKI guideline (1). Immediately at the end of the chosen exposure time, activity of the disinfectant was stopped by serial dilutions.

Since the undiluted test product was highly viscous, the first dilution step was performed directly in the test tube of the inactivation assay by addition of ice cold cell culture medium (9 ml).

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 (0.01 ml) test virus suspension, 0.1 ml (0.09 ml) interfering substance and 0.8 ml (0.9 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. As virus control for the 90 % assay, 0.1 part by volume of test virus suspension was mixed with 9.9 parts by volume of Aqua bidest. or with 0.9 parts by volume of FCS and nine 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, 30 und 60 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 were used in order to remove the cytotoxic agents according to instructions of the manufacturer. Virus controls without columns were included.

For the control of cell sensitivity 0.1 parts by volume Aqua bidest. or 0.09 part by volume of FCS and 0.01 part by volume Aqua bidest were mixed with 0.9 parts by volume of the of the product (PBS as reference) and 9 part of cell culture medium. 125 µl of this mixture were laid on a column. A serial dilution of the eluate was added to the permissive cell culture. After 1 h

at 37 °C the mixture was discharged and a comparative titration of the test virus suspension was performed on the pre-treated and non-pre-treated (PBS) cells as described above.
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 with 2 % FCS and 100 µl of each dilution were placed in 8 wells of a sterile polystyrene flat bottomed microtitre plate. 100 µl of fresh trypsinized *Vero cells* were added. 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.

6. Results

6.1 Determination of cytotoxicity

In parallel with the inactivation tests, the cytotoxicity of ProCare+ (90.0 %, 10.0 % and 1.0 %) and 0.7 % formaldehyde was measured. The formaldehyde solution was toxic for the *Vero cells* in the 1:100 dilutions. This corresponded to a $\log_{10}CD_{50}$/ml of 3.50 (Table 1).

Examinations also showed that ProCare+ was cytotoxic in the 1:1,000 dilutions. After introduction of the columns, the cytotoxicity of the undiluted product was reduced to a $\log_{10}CD_{50}$/ml of 2.50 to 3.50.

The 10.0 % solution was cytotoxic in the 1:100 dilutions. The 10.0 % solution still showed cytotoxicity in the 1:10 dilutions.

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

6.2 Virus-inactivating properties of formaldehyde control

Formaldehyde (0.7 %) reduced the vaccinia virus titre after 5 and 15 minutes by 0.75 ± 0.44 and 1.50 ± 0.44 $\log_{10}$ steps. After 30 and 60 minutes reduction factors of 2.13 ± 0.25 and ≥ 3.88 ± 0.41 were measured (Table 9).

6.3 Virus-inactivating properties of disinfectant

Results of inactivation assays are demonstrated in tables 2 to 9 (raw data see appendix).

ProCare+ was examined undiluted (90.0 %) and as 10.0 % and 1.0 % (demonstration of the non-active range) solutions. 30, 60 and 120 seconds were chosen as exposure times.

ProCare+ was active against vaccinia virus undiluted (90.0 %) within 30 seconds of exposure time. The reduction factors were ≥ 2.00 ± 0.33 and ≥ 2.25 ± 0.31 (assays without soil load) and ≥ 2.13 ± 0.18 and ≥ 2.38 ± 0.29 (assays with soil load), respectively (Tables 2, 3 and 4). The mean values are ≥ 2.13 ± 0.23 (assays without soil load) and ≥ 2.25 ± 0.17 (assays with soil load). Due to the cytotoxicity a reduction of 4 $\log_{10}$ steps could not be demonstrated.

After introduction of the columns the reduction factors were ≥ 4.00 ± 0.33 (assay without soil load) and ≥ 4.25 ± 0.23 (assay with soil load) (Tables 6 and 7). This corresponded to an inactivation of ≥ 99.99 %.

The 10.0 % solution was active against vaccinia virus within 120 seconds exposure time in the presence of FCS (RF ≥ 4.00 ± 0.25) (Table 8).

The 1.0 % solution was not able to reduce the virus titre within 120 seconds exposure time in the presence of FCS (Table 9).

- 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 01.08.2008) Hyg & Med, 33, 2008, 315-322

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

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

before treatment	Conc.	Interfering substance	Dilutions				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
test product	90.0%	Aqua bidest.	t	t	t	-	-
test product	90.0%	10.0% FCS	t	t	t	-	-
test product	10.0%	Aqua bidest.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	10.0%	10.0% FCS	t	t	-	-	-
test product	1.0%	Aqua bidest.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	1.0%	10.0% FCS	t	-	-	-	-
formaldehyde	0.7%	PBS	t	t	-	-	-
after treatment	**Conc.**	**Interfering substance**	**Dilutions**				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
test product	90.0%	Aqua bidest.	t	-	-	-	-
test product	90.0%	10.0% FCS	t	-	-	-	-
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.d.

t = cytotoxic n.d. = not done

Table 2: Inactivation of vaccinia virus by ProCare+ (90.0 %) 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	90.0%	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
test product	90.0%	9.0% FCS	≤4.50±0.00	≤4.50±0.00	≤4.50±0.00	n.d.	≥2.38±0.29	≥2.38±0.29	≥2.38±0.29	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.	9.0% FCS	n.d.	n.d.	n.d.	6.88±0.41	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.	9.0% 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 vaccinia virus by ProCare+ (90.0 %) 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	90.0%	Aqua bid.	≤4.50±0.00	≤4.50±0.00	n.d.	n.d.	≥2.00±0.33	≥2.00±0.33	n.a.	n.a.	≥ 30 s
test product	90.0%	9.0% 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.	6.50±0.46	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	9.0% 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.	9.0% 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 vaccinia virus by ProCare+ (90.0 %) and formaldehyde (0.7 %) in a quantitative suspension test at 20 °C (3rd 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	90.0%	Aqua bid.	≤4.50±0.00	n.d.	n.d.	n.d.	≥2.25±0.31	n.a.	n.a.	n.a.	≥ 30 s
test product	90.0%	9.0% FCS	≤4.50±0.00	n.d.	n.d.	n.d.	≥2.13±0.18	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.	6.75±0.44	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	9.0% FCS	n.d.	n.d.	n.d.	6.63±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.	9.0% 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 vaccinia virus by ProCare+ (90.0 %) 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	90.0%	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
test product	90.0%	9.0% FCS	≤2.50±0.00	n.d.	n.d.	n.d.	≥3.63±0.32	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.	9.0% FCS	n.d.	n.d.	n.d.	6.13±0.45	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.	9.0% 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 vaccinia virus by ProCare+ (90.0 %) 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	90.0%	Aqua bid.	≤2.50±0.00	n.d.	n.d.	n.d.	≥4.00±0.33	n.a.	n.a.	n.a.	30 s
test product	90.0%	9.0% 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.	6.50±0.46	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	9.0% 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.	9.0% 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 vaccinia virus by ProCare+ (90.0 %) and formaldehyde (0.7 %) in a quantitative suspension test at 20 °C (with columns) (3rd 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	90.0%	Aqua bid.	≤3.50±0.00	n.d.	n.d.	n.d.	≥3.13±0.18	n.a.	n.a.	n.a.	≥ 30 s
test product	90.0%	9.0% FCS	≤2.50±0.00	n.d.	n.d.	n.d.	≥4.25±0.23	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.	6.63±0.25	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	9.0% FCS	n.d.	n.d.	n.d.	6.75±0.33	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	7.38±0.25	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	7.63±0.25	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	9.0% FCS	n.d.	n.d.	n.d.	7.38±0.41	n.a.	n.a.	n.a.	n.a.	n.a.

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

Table 8: Inactivation of vaccinia virus by ProCare+ (10.0 %) 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	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	n.d.	≤3.63±0.25	≤3.50±0.00	n.d.	n.a.	≥3.88±0.43	≥4.00±0.25	n.a.	120 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.	10.0% FCS	n.d.	n.d.	n.d.	7.50±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 9: Inactivation of vaccinia virus by ProCare+ (1.0 %) 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	1.0%	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
test product	1.0%	10.0% FCS	n.d.	7.63±0.25	7.88±0.41	n.d.	n.a.	0.00±0.35	0.00±0.48	n.a.	> 120 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	6.88±0.37	6.13±0.37	5.50±0.00	≤4.75±0.33	0.75±0.44	1.50±0.44	2.13±0.25	≥3.88±0.41	> 60 min
virus control	n.a.	Aqua bid.	n.d.	n.d.	n.d.	7.63±0.25	n.a	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	10.0% FCS	n.d.	n.d.	n.d.	7.63±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