DR. BRILL + DR. STEINMANN
INSTITUTE FOR HYGIENE AND MICROBIOLOGY

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ZLG-AP-216.11.02

TEST REPORT

18/11/2020

Test report L20/1244MV.1

Evaluation of the effectiveness of

Vega Wipes General Cleaning and Disinfecting Wet Wipes

Test virus: modified vaccinia virus Ankara (MVA)

Method: based on EN 14476:2013+A2:2019 (3.0 g/l BSA)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine (phase 2/ step 1)

Sponsor:
Sasson Industies
Hazor St.
Industrial Park, Kidmat Galil
IL - ISRAEL

Norderoog 2, DE - 28259 Bremen
Tel.: +49 40-557631-0, Fax: +49 40-557631-11
info@brillhygiene.com, http://www.brillhygiene.com

DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

Sasson Industies
Hazor St.
Industrial Park, Kidmat Galil
IL - ISRAEL

Bremen, 18/11/2020

Expert opinion

Activity of Vega Wipes General Cleaning and Disinfecting Wet Wipes against modified vaccinia virus Ankara (MVA) in a quantitative suspension test based on EN 14476:2013+A2:2019 with 3.0 g/l BSA as interfering substance

This expert opinion is based on the test report L20/1244MV.1 dating 18/11/2020.

The virus-inactivating properties of the surface disinfectant Vega Wipes General Cleaning and Disinfecting Wet Wipes of Sasson Industries against modified vaccinia virus Ankara (MVA) were investigated by a quantitative suspension test based on EN 14476 with 3.0 g/l BSA as interfering substance.

According to this norm, a disinfectant or a disinfectant solution at a particular concentration is considered as having virus-inactivating properties if within the recommended exposure period the titre is reduced by ≥ 4 $\log_{10}$ (inactivation ≥ 99.99 %).

The surface disinfectant Vega Wipes General Cleaning and Disinfecting Wet Wipes was examined as 50.0 % solution at 20 °C. 5 minutes were chosen as exposure time. In summary, a virucidal activity against modified vaccinia virus Ankara (MVA) was measured as follows:

undiluted **5 minutes** **3.0 g/l BSA**

Dr. Jochen Steinmann

DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

Sasson Industies
Hazor St.
Industrial Park, Kidmat Galil
IL - ISRAEL

Bremen, 18/11/2020

Summary: Virus-inactivating properties (virucidal activity against enveloped viruses) of Vega Wipes General Cleaning and Disinfecting Wet Wipes of Sasson Industies based on EN 14476:2013+A2:2019 with 3.0 g/l BSA as interfering substance

This summary is based on the following test report of Dr. Brill + Partner GmbH for the surface disinfectant Vega Wipes General Cleaning and Disinfecting Wet Wipes produced by Sasson Industies:

modified vaccinia virus Ankara test report (L20/1244MV.1) dating 18/11/2020

The following concentration and exposure time are necessary for the inactivation of the test virus:

undiluted 5 minutes

in order to achieve a 4 $\log_{10}$ reduction (inactivation ≥ 99.99 %) with 3.0 g/l BSA as interfering substance in a quantitative suspension test based on EN 14476:2013+A2:2019.

After evaluation with modified vaccinia virus Ankara the surface disinfectant Vega Wipes General Cleaning and Disinfecting Wet Wipes can be declared as having **"virucidal activity against all enveloped viruses"** based on EN 14476:2013+A2:2019.

The declaration **"virucidal activity against all enveloped viruses"** covers all enveloped viruses (Annex A) like HBV, HCV, HIV as well as members of other virus families such as orthomyxoviridae (incl. all human influenza viruses), coronaviridae (like MERS-CoV, SARS-CoV-1 and SARS-CoV-2) and filoviridae including Ebola virus.

Dr. Jochen Steinmann

Extract from Annex A in EN 14476
Examples of viruses which may contaminate human medical instruments, hands, surfaces (*Enveloped viruses*)

NOTE This list is not exhaustive.

Blood

Filoviridae
Flavivirus
Herpesviridae
Hepatitis B virus (HBV)
Hepatitis C virus (HCV)
Hepatitis Delta virus (HDV)
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)

Respiratory tract

Coronavirus
Herpesviridae
Influenza Virus
Paramyxoviridae
Rubella Virus

Neural tissue, ear & nose, eye

Herpesviridae
Measles Virus
Human Immunodeficiency Virus (HIV)
Rabies Virus
Rubella Virus

Gastro-intestinal

Coronavirus

Skin, breast and/or milk

Herpesviridae
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Poxviridae

Spleen and lymph nodes (see also „Blood")

Human T Cell Leukemia Virus (HTLV)
Human Immunodeficiency Virus (HIV)

Dental procedure

Herpesviridae
Hepatitis B virus (HBV)
Hepatitis C Virus (HCV)
Hepatitis Delta Virus (HDV)
Human Immunodeficiency Virus (HIV)

Urogenital tract

Hepatitis B Virus (HBV)
Herpesviridae
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)

Reference:
Van Regenmortel MHV et al., Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses.
Academic Press, San Diego, 2000

1. Identification of test laboratory

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

2. Identification of sample

Manufacturer	Sasson Industries
Name of product	Vega Wipes General Cleaning and Disinfecting Wet Wipes
Confirmation no.	216168
Product diluent recommended by the manufacturer	-
Batch number	-
Product code	SC 230810.4
Application	surface disinfection
Production date	-
Expiry date	-
Active compound (s) (100 g)	0.16 % alkyl dimethyl benzyl ammonium chloride 0.16 % alkyl dimethyl ethylbenzyl ammonium chloride
Appearance, odour	clear, colorless liquid product specific
pH-values	undiluted: 6.44 (20 °C) 50.0 %: 7.28 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	04/08/2020

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880120)
- fetal calf serum (Thermo Fisher, article no. CH30160.02)
- 1.4 % formaldehyde solution (dilution of Roti®-Histofix 4 %, Carl Roth GmbH)
- Aqua bidest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)

- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153).

3.2 Virus and cells

The modified vaccinia virus Ankara (MVA) originated from Dr. Manteufel, Institut für Tierhygiene und Öffentliches Veterinärwesen, DE - 04103 Leipzig. Before inactivation assays, virus had been passaged four times in *BHK 21-cells* (Baby Hamster Kidney).

BHK 21-cells (passage 7) originated from the Friedrich-Löffler-Institut, Bundesforschungsinstitut für Tiergesundheit (formerly Bundesforschungsanstalt für Viruskrankheiten der Tiere, isle of Riems).

The cells were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

3.3 Apparatus, glassware and small items of equipment

- CO_2 incubator
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable and fixed-volume pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	50.0 %, 10.0 % and 1.0 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	5 minutes
Interfering substance	3.0 g/l bovine serum albumin (dirty conditions food area)
Procedure to stop action of disinfectant	immediate dilution
Diluent	Aqua bidest.
Stability of product in the mix with virus and interfering substance (50.0 % solution)	no clouding, no precipitation
Virus strain	modified vaccinia virus Ankara (MVA) (ATCC VR-1508)
Date of testing	29/10/2020 – 17/11/2020
End of testing	18/11/2020

5. Methods

5.1 Preparation of test virus suspension

To prepare the test virus suspension, *BHK 21-cells* were cultivated with MEM and 10 % or 2 % fetal calf serum.

Cells were infected with a multiplicity of infection of 0.1. After cells showed a cytopathic effect, they were subjected to a freeze/thaw procedure followed by a low speed centrifugation 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 is a ready to use product, but because of the cytotoxicity the test product was tested as 50.0 %, 10.0 % and 1.0 % solutions (1 part test virus suspension + 1 part interfering substance + 8 parts disinfectant). Due to the addition of interfering substance and test virus suspension the solutions had to be prepared by the factor 1.25.

These solutions were prepared with Aqua bidest. immediately before the inactivation tests.

5.3 Infectivity assay

Infectivity was determined as endpoint titration according to EN 5.5 transferring 0.1 ml of each dilution into eight wells of a microtitre plate to 0.1 ml of freshly splitted cells (10-15 x 10^3 cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3).

5.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to the EN 14476, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by 4 $\log_{10}$ steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99.99 %.

5.5 Inactivation assay (end point titration)

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

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8}.

Titrations of the virus control were performed at the beginning of the test and after the longest exposure time (EN 5.5.7). One part by volume of test virus suspension was mixed with one part interfering substance and eight parts by volume of WSH or Aqua bidest. (RTU products).

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

Inactivation tests were carried out in sealed test tubes in a water bath at 20 °C ± 1.0 °C. Aliquots were retained after appropriate exposure times and residual infectivity was determined.

5.6 Inactivation assay following the large volume plating method (LVP)

Following the large volume plating method (EN 5.5.4.3) the inactivation assays were further diluted 1:5,000 in cell culture medium. The total volume was added (without any further dilution) to the permissive cells. By introducing such a huge dilution it is possible to eliminate cytotoxicity of the test product in order to demonstrate a 4 $\log_{10}$ reduction of virus titre. Calculation of virus titre follows formula of Taylor or Poisson (EN B.3). This method is necessary for those products which demonstrate a great cytotoxicity.

12.5 µl of the inactivation assay were added to 62.5 ml medium and then the total volume was distributed in 6 microtitre plates (108 µl / well, 576 wells total).

5.7 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.8 Cell sensitivity to virus

For the control of cell sensitivity to virus two parts by volume of water were mixed with eight parts by volume of the lowest apparently non-cytotoxic dilution of the product. This mixture or PBS as control was added to a volume of double concentrated cell suspension. After 1 h at 37 °C the cells were centrifuged and re-suspended in cell culture medium (EN 5.5.4.2b).

Finally, a comparative titration of the test virus suspension was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

5.9 Control of efficacy for suppression of disinfectant's activity

Furthermore, a control of efficiency for suppression of disinfectant's activity was included (EN 5.5.5).

5.10 Reference virus inactivation test

As reference for test validation a 0.7 % formaldehyde solution according to EN 5.5.6 was included. 5, 15, 30 and 60 minutes were chosen as contact times. In addition, cytotoxicity of formaldehyde test solution was determined based on EN 5.5.6.2 with dilutions up to 10^{-5}.

6. Verification of the methodology

The following criteria as mentioned in EN 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of a ≥ 4 log_{10} reduction (maximal virus reduction ≥ 4.15 ± 0.38, LVP)

b) The test product (50.0 %) showed cytotoxicity in the 1:1,000 dilutions thus allowing the detection of a 4 log_{10} reduction of virus titre.

c) The difference of the logarithmic titre of the virus control minus the logarithmic titre of the test virus in the reference inactivation test (see EN 5.7b) was ≥ 1.63 ± 0.65 (between 0.75 – 3.5) after 5 min and ≥ 2.38 ± 0.41 (between 2.0 – ≥ 4.0) after 15 min for MVA.

d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) cells showed no significant difference (< 1 log_{10}; EN 5.7) of virus titre: 6.75 ± 0.35 (PBS, LVP) versus 6.38 ± 0.25 (1:5,000 dilutions of disinfectant as 50.0 % solution, LVP) log_{10} $TCID_{50}$/ml.

e) The control of efficacy for suppression of disinfectant's activity (50.0 %) showed a decrease of ≥ 2.00 (4.50 ± 0.00 versus 6.50 ± 0.35 log_{10} $TCID_{50}$/ml) and failed the requirement of the EN (≤ 0.5 log_{10}; EN 5.5.5.1). In these experiments at the end of the defined exposure time the test mixture was immediately diluted not 1:10 as described in the control of efficacy for suppression of disinfectant's activity but directly 1:5,000 (LVP) and the dilutions transferred to the cell culture. For this reason, this control is not relevant when using the LVP. Therefore, despite the insufficient control of efficacy for suppression of disinfectant's activity the assay is valid.

f) One concentration demonstrated a 4 log_{10} reduction and (at least) one concentration demonstrated a log_{10} reduction of less than 4.

Since all criteria according EN 5.7 were fulfilled, examination with MVA based on EN 14476 is valid.

7. Results

Results of examination are shown in tables 1 to 9. Tables 1 to 7 demonstrate the raw data, whereas tables 8 (a+b) and 9 give a summary of results.

The 10.0 % and 1.0 % solutions were not able to inactivate MVA within 5 minutes of exposure time with 3.0 g/l BSA as interfering substance (tables 1 and 2).

Since it was not possible to test the undiluted test product in an 80.0 % assay due to cytotoxicity with the end point dilution method and even with the large volume plating method, the tested concentration was reduced to 50.0 %. The mean virus titre was $\log_{10}$ $TCID_{50}$/ml = 6.69 ± 0.38 (table 6).

The test product as 50.0 % solution was active after 5 minutes of exposure time (table 7). Since no residual virus was found in 576 cell culture units at this time point, the result according to the formula of Poisson was ≤ 2.54 $\log_{10}$ $TCID_{50}$. The reduction factor was therefore ≥ 4.15 ± 0.38 (6.69 ± 0.38 $\log_{10}$ $TCID_{50}$ minus ≤ 2.54 $\log_{10}$ $TCID_{50}$).

8. Conclusion

The surface disinfectant Vega Wipes General Cleaning and Disinfecting Wet Wipes tested as 50.0 % solution demonstrated activity against MVA after an exposure time of 5 minutes with 3.0 g/l BSA as interfering substance. Therefore, the surface disinfectant Vega Wipes General Cleaning and Disinfecting Wet Wipes can be declared as active against MVA as follows:

undiluted 5 minutes 3.0 g/l BSA

Bremen, 18/11/2020

- Dr. Britta Becker -
Head of Laboratory

- Dr. Dajana Paulmann -
Scientific Project Manager

9. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBI. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBI. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assays.

10. Records to be maintained

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

11. Literature

1. EN 14476:2013+A2:2019: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)

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

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

Appendix:

Legend to the Tables

Table 1: Raw data for Vega Wipes General Cleaning and Disinfecting Wet Wipes (10.0 %) tested against MVA

Table 2: Raw data for Vega Wipes General Cleaning and Disinfecting Wet Wipes (1.0 %) tested against MVA

Table 3: Raw data for formaldehyde solution (0.7 %) tested against MVA

Table 4: Raw data for control of efficacy for suppression of disinfectant's activity (50.0 %)

Table 5: Raw data (MVA) for cell sensitivity (50.0 %) (LVP)

Table 6: Determination of virus titre (LVP)

Table 7: Inactivation of MVA by Vega Wipes General Cleaning and Disinfecting Wet Wipes (50.0 %) (5 minutes) (LVP)

Table 8 (a+b): Summary of results (end point dilution method) with Vega Wipes General Cleaning and Disinfecting Wet Wipes and MVA

Table 9: Summary of results (LVP) with Vega Wipes General Cleaning and Disinfecting Wet Wipes and MVA

Legend to the Figures

Figure 1: Virus-inactivating properties of Vega Wipes General Cleaning and Disinfecting Wet Wipes (50.0 %) (LVP)

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

Table 1: Raw data for Vega Wipes General Cleaning and Disinfecting Wet Wipes (10.0 %) tested against MVA at 20 °C (quantal test; 8 wells) (#6972)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0 %	3.0 g/l BSA	5	n.d.	n.a.	n.a.	4444 4444	0202 3000	0000 0000	0000 0000	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	10.0 %	3.0 g/l BSA	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	3.0 g/l BSA	0	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	0002 0004	0000 0200	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	3324 0344	0000 0030	0000 0000	0000 0000	n.d.

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

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 2: Raw data for Vega Wipes General Cleaning and Disinfecting Wet Wipes (1.0 %) tested against MVA at 20 °C (quantal test; 8 wells) (#6972)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	1.0 %	3.0 g/l BSA	5	n.d.	n.a.	n.a.	4444 4444	4404 4204	0040 0433	0000 0000	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	1.0 %	3.0 g/l BSA	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	3.0 g/l BSA	0	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	0002 0004	0000 0200	0000 0000	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	3324 0344	0000 0030	0000 0000	0000 0000	n.d.

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

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 3: Raw data for formaldehyde solution (0.7 %) tested against MVA at 20 °C (quantal test; 8 wells) (#6972)

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	2010 2200	0200 0020	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0300 3000	0000 0020	0000 0000	n.d.

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

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 4: Raw data for control of efficacy for suppression of disinfectant's activity (50.0 %) (#6972)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	3.0 g/l BSA	n.d.	n.a.	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
corresponding virus control	3.0 g/l BSA	4444 4444	4444 4444	4444 4444	4444 4444	3324 0344	0000 0030	0000 0000	0000 0000	n.d.

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

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 5: Raw data (MVA) for cell sensitivity (50.0 % solution) (#6972) (LVP)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4432 4331	0200 0000	0000 0030	0000 0000	n.d.
test product	1:5,000	4444 4444	4444 4444	4444 4444	4444 4444	2404 4214	0000 0000	0000 0000	0000 0000	n.d.

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

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 6: Determination of virus titre (LVP) at 20 °C (#6972)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
(beginning of test)	3.0 g/l BSA	4444 4444	4444 4444	4444 4444	4444 4444	4440 4444	0002 0004	0000 0200	0000 0000	n.d.
1st control	3.0 g/l BSA	4444 4444	4444 4444	4444 4444	4444 4444	3324 0344	0000 0030	0000 0000	0000 0000	n.d.
2nd control	3.0 g/l BSA	4444 4444	4444 4444	4444 4444	4444 4444	4434 4344	2030 0000	4000 0000	0000 0000	n.d.

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

0 = no virus present
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 7: Inactivation of MVA by Vega Wipes General Cleaning and Disinfecting Wet Wipes (50.0 %) at 20 °C (5 minutes) (LVP, 1:5,000) (#6972)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
3.0 g/l BSA	plate 1/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 2/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 3/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 4/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 5/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 6/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

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

Table 8a: Summary of results (end point dilution method) with Vega Wipes General Cleaning and Disinfecting Wet Wipes and MVA

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after …min
				1	5	15	30	60	
test product	10.0 %	3.0 g/l BSA	3.50	n.d.	5.88±0.37	n.d.	n.d.	n.d.	> 5 (RF = 0.63±0.51)
test product	1.0 %	3.0 g/l BSA	2.50	n.d.	6.75±0.50	n.d.	n.d.	n.d.	> 5 (RF = 0.00±0.61)

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

Table 8b: Summary of results (end point dilution method) with Vega Wipes General Cleaning and Disinfecting Wet Wipes and MVA

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after … min
				0	5	15	30	60	
formaldehyde	0.7 % (w/v)	PBS	4.50	n.d.	≤ 5.25±0.50	≤ 4.50±0.00	≤ 4.50±0.00	≤ 4.50±0.00	≥ 15 (RF ≥ 2.38±0.41)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	6.88±0.41	n.a.
virus control (+ suppression)	n.a.	3.0 g/l BSA	n.a.	6.75±0.48	n.d.	n.d.	n.d.	6.50±0.35	n.a.
suppression control	50.0 %	3.0 g/l BSA	n.d.	n.d.	n.d.	n.d.	≤ 4.50±0.00	n.d.	n.a.

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

Table 9: Summary of results (LVP, 1:5,000) with Vega Wipes General Cleaning and Disinfecting Wet Wipes and MVA

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after …min
				0	1	5	15	60	
test product	50.0 %	3.0 g/l BSA	n.a.	n.d.	n.d.	≤ 2.54	n.d.	n.d.	5 (RF ≥ 4.15±0.38)
virus control	n.a.	3.0 g/l BSA	n.a.	6.75±0.48	n.d.	n.d.	n.d.	6.50±0.35 6.88±0.41 (Ø6.69±0.38)	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.35	n.a.
sens. product	50.0 % → 1:5,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.38±0.25	n.a.

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

Figure 1: Virus-inactivating properties of Vega Wipes General Cleaning and Disinfecting Wet Wipes (50.0 %) (LVP)

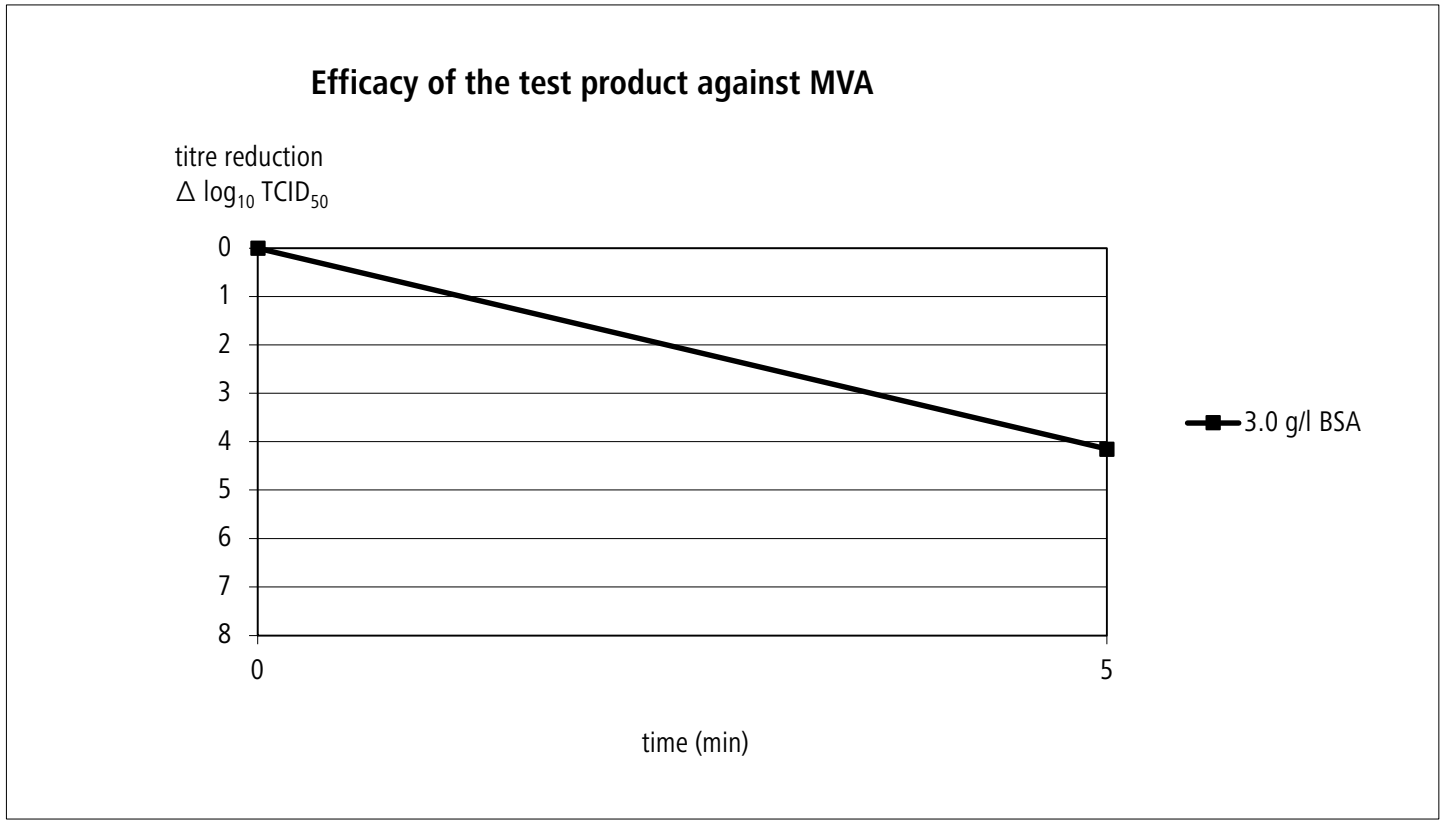

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)