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

Advanced Precision Laboratories BVBA
Brandstraat 12A
BE – 9160 Lokeren

Bremen, 24/09/2018

Summary: Virus-inactivating properties of the surface disinfectant NERTA OMNIKYLL of Advanced Precision Laboratories BVBA in a quantitative suspension test according EN 14675 in veterinary medicine (PT 3)

This summary is based on the following test reports of Dr. Brill + Partner GmbH for the surface disinfectant NERTA OMNIKYLL produced by Advanced Precision Laboratories BVBA:

ECBO virus test report (L18/0256E.2) dating 13/06/2018

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

10 °C 1.75 % 30 minutes

in order to achieve a four $\log_{10}$ reduction (inactivation ≥ 99.99 %) with high protein load in a quantitative suspension test according to EN 14675.

This European Standard EN 14675 applies to products that are used in the veterinary area i.e. in the breeding, husbandry, production, transport and disposal of all animals except when in the food chain following death and entry to the processing industry.

Therefore, after successful experiments with the ECBO virus type 1 NERTA OMNIKYLL is also effective against other viruses from veterinary medicine like the African swine fever virus (ASFV) or the avian influenza A virus.

Dr. Jochen Steinmann

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NOTARIS - ANTWERPEN - Pieter HERMAN

Sworn to and subscribed before me this 24/9/2018
Pieter HERMAN
Notary public at Antwerp

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
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INTERNET WWW.BRILLHYGIENE.COM

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

Advanced Precision Laboratories BVBA
Brandstraat 12A
BE – 9160 Lokeren

Bremen, 13/06/2018

Expert opinion

Activity of NERTA OMNIKYLL against ECBO virus in a quantitative suspension test according to EN 14675:2015

This expert opinion is based on the test report L18/0256E.2 dating 13/06/2018.

The virus-inactivating properties of the surface disinfectant NERTA OMNIKYLL of Advanced Precision Laboratories BVBA against bovine enterovirus type 1 (ECBO virus strain LCR-4) were investigated by a quantitative suspension test according to EN 14675:2015 with high-level soiling.

According to the EN 14675:2015, 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 %).

NERTA OMNIKYLL was examined as 1.75 %, 1.25 % and 0.75 % solutions at 10 °C. 30 minutes were chosen as exposure time. In summary, a virucidal activity against ECBO virus type 1 was measured as follows:

10 °C **1.75 %** **30 minutes** **high-level soiling (10.0 g/l BSA + 10.0 g/l yeast extract)**

Dr. Jochen Steinmann

Notaris Pieter HERMAN BVBA
www.notaris-herman.be
Van Breestraat 33 - 2018 Antwerpen
Tel.: 03/233.17.66 - Fax: 03/231.59.75
BTW: BE 0658.869.827

Sworn to and subscribed before me this 20/6/2018
Pieter HERMAN
Notary public at Antwerp

DR. BRILL + DR. STEINMANN

13/06/2018

Test report L18/0256E.2

Evaluation of the effectiveness of
NERTA OMNIKYLL

Test virus:	bovine enterovirus type 1 (Entero Cytopathogenic Bovine Orphan - ECBO)
Method:	EN 14675:2015 (10 °C) (high-level soiling)
	Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area – Test method and requirements (phase 2, step 1)

Sponsor:
Advanced Precision Laboratories BVBA
Brandstraat 12A
BE – 9160 Lokeren

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

1. Introduction

The objective of this study was to evaluate the virus-inactivating properties of the surface disinfectant NERTA OMNIKYLL against bovine enterovirus type 1 (Entero Cytopathogenic Bovine Orphan – ECBO) using a quantitative suspension assay according to EN 14675 (1).

2. Identification of test laboratory

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

3. Identification of sample

Manufacturer	Advanced Precision Laboratories BVBA
Name of product	NERTA OMNIKYLL
Confirmation no.	204995
Product diluent recommended by the manufacturer	-
Batch number	ENT-160525
Application	surface disinfection
Production date	-
Expiry date	-
Active compound (s) (100 g)	Didecyldimethylammonium chloride 20-30 % glutaral, glutaraldehyde, 1,5-pentanedial 10-20 % Propan-2-ol 1-10 % Fatty alcohol ethoxylated 1-10 %
Appearance, odour	clear, slightly brownish liquid product specific
pH-values	undiluted: 4.96 (20 °C) 1.75 %: 6.91 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	24/04/2018

4. Materials

4.1 Culture medium and reagents

- Eagle's Minimum Essential Medium with Earle's BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880121)
- fetal calf serum (Biochrom AG, article no. S 0115)
- 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).

4.2 Virus and cells

The ECBO virus was obtained from Dr. W. Herbst, Institut für Hygiene und Infektionskrankheiten der Tiere, Justus-Liebig-Universität Giessen.

Before the inactivation assays, the virus had been passaged 3 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).

4.3 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)
- 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).

5. Experimental conditions

Test temperature	20 °C ± 1.0 °C and 10 °C ± 1.0 °C
Concentration of test product	1.75 %, 1.25 %, 0.75 %, 0.175 % and 0.01 % (demonstration of non-active range) solutions
Contact times	30 minutes
Interfering substance	10 g/l BSA + 10 g/l yeast extract (high-level soiling)
Procedure to stop action of disinfectant	immediate dilution
Stability of product in the mix with virus and interfering substance (1.75 % solution)	medium clouding, strong precipitation
Diluent	water of standardised hardness (WSH)
Virus strain	bovine enterovirus type 1 (ECBO virus strain LCR-4) (ATCC VR-248)
Date of testing	25/04/2018 – 13/06/2018
End of testing	13/06/2018

6. Methods

6.1 Preparation of test virus suspension

For the preparation of the test virus suspension, *eKL cells*, which were cultivated with Eagle's Minimum Essential Medium (EMEM) supplemented with L-glutamine, sodium pyruvate and 2 % fetal calf serum (FCS), were infected with ECBO virus (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 in order to sediment cell debris. After aliquotation, test virus suspension was stored at –80 °C.

6.2 Preparation of disinfectant (dilutions)

The test product was tested as 1.75 %, 1.25 %, 0.75 %, 0.175 % and 0.01 % 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 WSH immediately before the inactivation tests.

6.3 Infectivity assay

Infectivity was determined by means of end point dilution method using the microtitre process. For this, 100 µl aliquots of the samples, which had been serially diluted with ice-cold EMEM were transferred to eight cups of a sterile polystyrol 96-well microtitre plate with a preformed monolayer of *KOP-R cells* (placed in each well on the previous day; 100 µl aliquots with approx. 1.5 x 10^4 cells). Incubation took place at 37°C in a CO_2 incubator (5 % CO_2 content) for 9 days. Finally, cultures were observed for cytopathic effects with a reversed microscope and the infective dose $TCID_{50}$/ml was calculated with the method of Kärber (2) and Spearman (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 pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

6.4 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 14675, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by four $\log_{10}$ steps within the recommended exposure period.

6.5 Inactivation assays (end point titration)

Investigations for determination of virucidal activity followed to EN 5.7. The test product was examined as 1.75 %, 1.25 %, 0.75 %, 0.175 % and 0.01 % (demonstration of non-active range) solutions at 10 °C according to EN 14675. 30 minutes were chosen as contact time.

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

Before the addition of the test product, test virus suspension and interfering substance were mixed and incubated for 2 minutes at 10 °C.

Titration of the virus control was performed at a contact time of 30 min (EN 14675). 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 10 °C ± 1.0 °C and 20 °C ± 1.0 °C. Aliquots were retained after appropriate exposure times and residual infectivity was determined.

6.6 Inactivation assay following the large volume plating method

Following the large volume plating method (4) the inactivation assays were further diluted 1:1,000 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 (5, 6). This method is necessary for those products which demonstrate a great cytotoxicity.

62.5 µl of the inactivation assays were added to 62.5 ml EMEM (total dilution of 1:1,000) and then the total volume was distributed in 6 microtitre plates (108 µl / well, 576 wells total). After 9 days of inoculation cultures were observed for cytopathic effects.

The calculation of virus titre without residual virus followed the formula of Poisson:

$$c = \ln p / -V$$

c = number of virus particles

p = the probability to find no virus. The probability to find no virus should not greater than 5 % (p=0.05). By doing so, the number of virus particles can be calculated with a probability of 95 %.

V = test volume (ml)

The titre to be used for calculating the reduction factor (RF) was finally calculated as followed: the determined number of virus particle is first converted with the aid of the dilution factor in the number of particle per ml. Subsequently, the

numbers of particles per ml have to be converted in the tissue culture infectious dose per ml ($TCID_{50}$/ml) (1.0 $TCID_{50}$ corresponds to 0.69 infectious virus particles). The common logarithm of this value results in the virus titre (log_{10} $TCID_{50}$/ml) used for calculating the reduction factor (RF).

In assays with residual virus, formula according to Taylor was used for calculating the virus titre:

$$c/ml = \frac{D}{Vw} \times \left(-\ln \frac{n - n_p}{n}\right)$$

c = number of virus particles

D = dilution

V_W = volume per well

n = number of inoculated wells

n_p = number of virus-positive wells

Finally, the number of virus particles is converted to the logarithmic titre ($log_{10}TCID_{50}$/ml).

6.7 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN B.1 a. Values are given as log_{10} CD_{50}/ml (in analogy to log_{10} $TCID_{50}$/ml).

6.8 Cell sensitivity to virus

For the control of cell sensitivity two parts WSH was mixed with eight parts disinfectant (PBS as control). A non-toxic dilution of this mixture was added to a microtitre plate with a preformed monolayer of *KOP-R 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.

6.9 Control of efficacy for suppression of disinfectant's activity

Furthermore, a control of efficiency for suppression of disinfectant's activity was included (EN 5.8).

6.10 Reference virus inactivation test

A 0.7 (w/v) % formaldehyde solution was included as reference for test validation following EN B.2. Contact times were 5, 15, 30 and 60 minutes. In addition, cytotoxicity of formaldehyde test solution was determined with dilutions up to 10^{-5}.

7. Verification of the methodology

The following criteria as mentioned in EN 6.4 were fulfilled:

a) The titre of the test virus suspension allowed the determination of a four log_{10} reduction (see EN 6.4 a) (maximum detectable virus inactivation: 4.10, LVP).

b) 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 6.4 b) was ≥ 1.38 (between 0.5 and 2.5) after 30 minutes exposure time with ECBO virus.

c) The test product (1.75 %) was cytotoxic in the 1:100 dilutions (see EN 6.4 c) thus allowing demonstrating a 4 log_{10} reduction in virus titre with the LVP method.

d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (medium) *KOP-R cells* showed an acceptable difference (< 1.0 log_{10}; see EN 6.4 d) of virus titres: 6.88 (medium) versus 6.38 (disinfectant, 1.75 % solution, LVP) log_{10} $TCID_{50}$/ml.

e) The control of efficacy for suppression of disinfectant's activity (1 75 %) showed no decrease (≤ 0.5 log_{10}; EN 5.8) in virus titre (5.38 versus 5.75 log_{10} $TCID_{50}$/ml).

f) One concentration demonstrated a four log_{10} reduction and (at least) one concentration demonstrated a log_{10} reduction of less than 4 (see EN 6.4 f).

Since all criteria according to EN 6.4 were fulfilled, examination with ECBO virus according to EN 14675:2015 is valid.

8. Results

Results of examination are shown in tables 1 to 12. Tables 1 to 10 demonstrate the raw data, whereas tables 11 (a + b) and 12 give a summary of results.

Examining the test product as 1.75 % solution, no residual virus was found after 30 minutes in this quantitative suspension test using the end point dilution method at 10 °C (table 1). The reduction factor was ≥ 2.38 at this time point.

Due to the high cytotoxicity of the test product, a reduction of 4 log_{10}-steps could not be shown and method the large volume plating method (LVP) was introduced.

Testing the product as 1.25 %, 0.75 %, 0.175 % and 0.01 % solutions, no activity was found after 30 minutes (table 2, 3, 4 and 5).

Due to the high cytotoxicity of the test product the large volume plating method (LVP) was introduced testing the 1.75 % solution. The mean virus titre (twofold assay) was log_{10} $TCID_{50}$/ml = 5.94 (table 9).

The test product as 1.75 % solution was active after 30 minutes of exposure time at 10 °C (table 10). No residual virus was found in 576 cell culture units. The result according to the formula of Poisson was ≤ 1.84. The reduction factor was therefore ≥ 4.10 (5.94 log_{10} $TCID_{50}$ minus ≤ 1.84 log_{10} $TCID_{50}$) after 30 minutes of exposure time. This corresponded to an inactivation of ≥ 99.99 %.

9. Conclusion

The surface disinfectant NERTA OMNIKYLL tested as 1.75 % solution at 10 °C demonstrated activity against ECBO virus after an exposure time of 30 minutes with high-level soiling.

Therefore, the surface disinfectant NERTA OMNIKYLL can be declared as active against ECBO virus as follows:

10 °C 1.75 % 30 minutes high-level soiling

Bremen, 13/06/2018

- Dr. Britta Becker -
Head of Laboratory

- Dr. Dajana Paulmann
Scientific Project Manag

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10. Literature

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Appendix:

Table 1: Raw data of NERTA OMNIKYLL (1.75 %) tested against ECBO virus at 10 °C (quantal test; 8 wells) (#5510)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	1.75 %	high-level soiling	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
Test product cytotoxicity	1.75 %	high-level soiling	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	high-level soiling	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	4444 4444	4444 4444	4444 4444	4444 4444	0440 0040	0000 0000	0000 0000	0000 0000	0000 0000

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

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

Table 2: Raw data of NERTA OMNIKYLL (1.25 %) tested against ECBO virus at 10 °C (quantal test; 8 wells) (#5510)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	1.25 %	high-level soiling	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	tttt tttt	tttt tttt	0040 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
Test product cytotoxicity	1.25 %	high-level soiling	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	high-level soiling	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	4444 4444	4444 4444	4444 4444	4444 4444	0440 0040	0000 0000	0000 0000	0000 0000	0000 0000

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

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

Table 3: Raw data of NERTA OMNIKYLL (0.75 %) tested against ECBO virus at 10 °C (quantal test; 8 wells) (#5510)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	0.75 %	high-level soiling	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	tttt tttt	tttt tttt	4444 4444	4000 0040	0440 0040	0000 0000	0000 0000	n.d.	n.d.
Test product cytotoxicity	0.75 %	high-level soiling	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	high-level soiling	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	4444 4444	4444 4444	4444 4444	4444 4444	0440 0040	0000 0000	0000 0000	0000 0000	0000 0000

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

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

Table 4: Raw data of NERTA OMNIKYLL (0.175 %) tested against ECBO virus at 10 °C (quantal test; 8 wells) (#5533)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	0.175 %	high-level soiling	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	tttt tttt	4444 4444	4444 4444	4444 0444	4004 4440	0000 0000	0000 0000	n.d.	n.d.
Test product cytotoxicity	0.175 %	high-level soiling	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	high-level soiling	0	4444 4444	4444 4444	4444 4444	4444 4444	0000 0040	0000 0400	0000 0000	0000 0000	0000 0000
			30	4444 4444	4444 4444	4444 4444	4444 4444	0040 4000	0400 0004	0000 0000	0000 0000	0000 0000

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

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

Table 5: Raw data of NERTA OMNIKYLL (0.01 %) tested against ECBO virus at 10 °C (quantal test; 8 wells) (#5533)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Test product	0.01 %	high-level soiling	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	tttt tttt	4444 4444	4444 4444	4444 4444	0000 0004	0000 0440	0000 0000	n.d.	n.d.
Test product cytotoxicity	0.01 %	high-level soiling	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	high-level soiling	0	4444 4444	4444 4444	4444 4444	4444 4444	0000 0040	0000 0400	0000 0000	0000 0000	0000 0000
			30	4444 4444	4444 4444	4444 4444	4444 4444	0040 4000	0400 0004	0000 0000	0000 0000	0000 0000

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

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

Table 6: Raw data of formaldehyde solution (0.7 %) tested against ECBO virus at 20 °C (quantal test; 8 wells) (#5533)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Formaldehyde	0.7 % (m/V)	medium	5	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
Formaldehyde cytotoxicity	0.7 % (m/V)	medium	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
Virus control	n.a.	medium	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	4444 4444	4444 4444	4444 4444	4444 4444	0404 0004	0000 0000	0000 0000	0000 0000	0000 0000

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

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

Table 7: Raw data for control of efficacy for suppression of disinfectant's activity (1.75 %) (#5533)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	high-level soiling	tttt tttt	tttt tttt	4444 4444	4404 4444	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
corresponding virus control	high-level soiling	4444 4444	4444 4444	4444 4444	4444 4444	0000 4040	0000 0000	0000 0000	0000 0000	0000 0000

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

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

Table 8: Raw data (ECBO virus) for cell sensitivity (1.75 %) (LVP) (#5546)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
medium	-	4444 4444	4444 4444	4444 4444	4444 4444	4044 4444	0004 0404	4000 0000	0000 0000	n.d.
test product	1:1,000	4444 4444	4444 4444	4444 4444	4444 4444	4440 0444	0000 0004	0000 0000	0000 0000	n.d.

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

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

Table 9: Determination of virus titre (LVP) at 10 °C (#5533)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
1st control	high-level soiling	4444 4444	4444 4444	4444 4444	4444 4444	0040 4000	0400 0004	0000 0000	0000 0000	n.d.
2nd control	high-level soiling	4444 4444	4444 4444	4444 4444	4444 4444	0004 0440	0000 0000	0000 0000	0000 0000	n.d.

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

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

Table 10: Inactivation of ECBO virus by NERTA OMNIKYLL (1.75 %) at 10 °C (LVP, 1:1,000) (30 min) (#5533)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
high-level soiling	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 11a: Summary of results (end point dilution method) with NERTA OMNIKYLL and ECBO virus

Product*	Con-centration	Interfering substance	Temperature	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ... min
					1	5	15	30	60	
product (1)	1.75 %	high-level soiling	10 °C	3.50	n.d.	n.d.	n.d.	≤ 3.50	n.d.	≥ 30 (RF ≥ 2.38)
product (1)	1.25 %	high-level soiling	10 °C	3.50	n.d.	n.d.	n.d.	≤ 3.63	n.d.	> 30 (RF ≥ 2.25)
product (1)	0.75 %	high-level soiling	10 °C	3.50	n.d.	n.d.	n.d.	5.13	n.d.	> 30 (RF = 0.75)
product (2)	0.175 %	high-level soiling	10 °C	2.50	n.d.	n.d.	n.d.	6.00	n.d.	> 30 (RF = 0.00)
product (2)	0.01 %	high-level soiling	10 °C	2.50	n.d.	n.d.	n.d.	5.88	n.d.	> 30 (RF = 0.13)

*The number in brackets gives the number of the corresponding virus control, see Table 11b

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

Table 11b: Summary of results (end point dilution method) with NERTA OMNIKYLL and ECBO virus

Product	Con-centration	Interfering substance	Temperature	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ... min
					0	5	15	30	60	
formaldehyde	0.7 % (m/V)	medium	20 °C	4.50	n.d.	≤ 4.50	≤ 4.50	≤ 4.50	≤ 4.50	≥ 5.0
virus control	n.a.	medium	20 °C	n.a.	n.d.	n.d.	n.d.	5.88	n.d.	n.a.
virus control (1)	n.a.	high-level soiling	10 °C	n.a.	n.d.	n.d.	n.d.	5.88	n.d.	n.a.
virus control (2)	n.a.	high-level soiling	10 °C	n.a.	5.75	n.d.	n.d.	6.00	n.d.	n.a.
virus control (3) (suppression)	n.a.	high-level soiling	20 °C	n.a.	n.d.	n.d.	n.d.	5.75	n.d.	n.a.
suppression control	1.75 %	high-level soiling	n.a.	3.50	n.d.	n.d.	n.d.	5.38	n.d.	n.a.

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

Table 12: Summary of results (LVP, 1:1,000) with NERTA OMNIKYLL and ECBO virus

Product	Con-centration	Interfering substance	Temperature	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min						> 4 log_{10} reduction after ... min
					0	1	5	15	30	60	
product	1.75 %	high-level soiling	10 °C	n.a.	n.d.	n.d.	n.d.	n.d.	≤ 1.84	n.d.	30 (RF ≥ 4.10)
virus control	n.a.	high-level soiling	10 °C	n.a.	n.d.	n.d.	n.d.	n.d.	6.00 5.88 (Ø 5.94)	n.d.	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	6.88	n.a.
sens. product	1.75 % → 1:1,000	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	6.38	n.a.

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