

B. Braun Medical AG  
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Bremen, 26/04/2017

## Expert opinion

Activity of Hexaquart XL against modified vaccinia virus Ankara (MVA) in a quantitative suspension test based on EN 14476:2013+A1:2015 under dirty conditions

This expert opinion is based on the test report L17/0049MV.1 dating 26.04.2017.

The virus-inactivating properties of the surface disinfectant Hexaquart XL of B. Braun Medical AG against modified vaccinia virus Ankara (MVA) were investigated by a quantitative suspension test based on EN 14476 under dirty conditions.

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  $\geq 4 \log_{10}$  (inactivation  $\geq 99.99\%$ ).

Hexaquart XL was examined as 1.0 % and 0.5 % solutions at 20 °C. 5 and 15 minutes were chosen as exposure time. After 5 minutes exposure time the virus titre was decreased by  $\geq 4 \log_{10}$  steps testing the 1.0 % solution. Therefore, a virucidal activity against modified vaccinia virus Ankara (MVA) was measured as follows:

**1.0 %    5 minutes    dirty conditions**

  
Dr. Jochen Steinmann

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Bremen, 26. April 2017

## Gutachten

Wirksamkeit von Hexaquart XL gegenüber dem Modifizierten Vacciniavirus Ankara (MVA) im quantitativen Suspensionsversuch in Anlehnung an die EN 14476:2013+A1:2015 unter hoher Belastung

Dieses Gutachten basiert auf dem Prüfbericht L17/0049MV.1 vom 26.04.2017.

Das Flächendesinfektionsmittel Hexaquart XL der B. Braun Medical AG wurde gemäß Auftrag auf seine virusinaktivierenden Eigenschaften gegenüber dem Modifizierten Vacciniavirus Ankara (MVA) in Anlehnung an die EN 14476 unter hoher Belastung untersucht.

In der EN 14476 wird dann von einer Virus-Wirksamkeit eines Desinfektionsmittels ausgegangen, wenn nach einer bestimmten Einwirkzeit eine Reduktion des initialen Virustiters um  $\geq 4 \log_{10}$  Stufen (Inaktivierung  $\geq 99,99\%$ ) erfolgt ist.

Das Flächendesinfektionsmittel Hexaquart XL wurde als 1,0 %ige und 0,5 %ige Lösungen bei 20 °C untersucht. Die Einwirkzeiten betrugen 5 und 15 Minuten. Nach 5 Minuten war im Ansatz mit der 1,0 %igen Lösung eine ausreichende Reduktion des Virustiters nachweisbar. Deshalb ergibt sich eine Wirksamkeit gegenüber dem MVA wie folgt:

**1,0 %    5 Minuten    hohe Belastung**

  
Dr. Jochen Steinmann



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



26/04/2017

## Test report L17/0049MV.1

### Evaluation of the effectiveness of Hexaquart XL

Test virus: modified vaccinia virus Ankara (MVA)

Method: based on EN 14476:2013+A1:2015 (dirty conditions)

quantitative suspension test for the evaluation  
of virucidal activity of chemical disinfectants and  
antiseptics used in human medicine

#### Sponsor:

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## 1. Identification of test laboratory

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

## 2. Identification of sample

Manufacturer	B. Braun Medical AG
Name of product	Hexaquart XL
Product diluent recommended by the manufacturer	-
Batch number	R-40_25102016
Application	surface disinfection
Production date	-
Expiry date	-
Active compound (s) (100 g)	6.0 % Didecyldimethylammoniumchlorid 9.9 % N,N-Bis(3-Aminopropyl)dodecylamin
Appearance, odour	clear, reddish liquid product specific
pH-values	undiluted: 11.6 (20 °C) 1.0 %: 10.25 (20 °C) 0.5 %: 9.97 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	02/02/2017

## 3. Materials

### 3.1 Culture medium and reagents

- Eagle's Minimum Essential Medium with Hank's BSS (MEM, Biozym Scientific GmbH, catalogue no. 880144)
- 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)

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- sheep erythrocytes (Fiebig Nährstofftechnik).

### 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 passed three times in *BHK 21-cells* (Baby Hamster Kidney).

*BHK 21-cells* (passage 118) 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<sub>2</sub> 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)
- Polyesterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

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#### 4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	1.0 %, 0.5 % and 0.1 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	5, 15 and 30 minutes
Interfering substance	3.0 g/l bovine serum albumin + 3.0 g/l erythrocytes (dirty conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water of standardised hardness (WSH)
Stability of product in the mix with virus and interfering substance (1.0 % solution)	medium clouding, strong precipitation
Virus strain	modified vaccinia virus Ankara (MVA) (ATCC VR-1508)
Date of testing	02/02/2017 – 26/04/2017
End of testing	26/04/2017

#### 5. Methods

##### 5.1 Preparation of test virus suspension

For preparation of 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 was tested as 1.0 %, 0.5 % and 0.1 % (demonstrating of non-active range) solutions. 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.

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### 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 trypsinised *BHK 21-cells* ( $10\text{--}15 \times 10^3$  cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO<sub>2</sub>-atmosphere. The cytopathic effect was read by using an inverted microscope after six days. Calculation of the infective dose TCID<sub>50</sub>/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

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

meaning

$X_0$  = log<sub>10</sub> 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.

### 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<sub>10</sub> steps within the recommended exposure period. This corresponds to an inactivation of  $\geq 99.99\%$ .

### 5.5 Inactivation assay (end point titration)

Determination of virucidal activity has been carried out based on EN 5.5. The test product was examined as 1.0 %, 0.5 % and 0.1 % (demonstration of non-active range) solutions in WSH at 20 °C based on EN 14476. 5, 15 and 30 minutes were chosen as contact times.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10<sup>-8</sup>.

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Titration 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  $20\text{ }^{\circ}\text{C} \pm 1.0\text{ }^{\circ}\text{C}$ . 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 (4) 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 (5, 6). This method is necessary for those products which demonstrate a great cytotoxicity.

12.5 µl of the inactivation assays were added to 62.5 ml medium (total dilution of 1:5,000) and then the total volume was distributed in 6 microtitre plates (108 µl / well, 576 wells total). After 6 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 be 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 ( $\text{TCID}_{50}/\text{ml}$ ) ( $1.0\text{ TCID}_{50}$

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corresponds to 0.69 infectious virus particles). The common logarithm of this value results in the virus titre ( $\log_{10}$  TCID<sub>50</sub>/ml) used for calculating the reduction factor (RF).

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

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

c = number of virus particles

D = dilution

V<sub>w</sub> = volume per well

n = number of inoculated wells

n<sub>p</sub> = number of virus-positive wells

For calculating the reduction factor using the formula according to Taylor the number of virus particles is converted to the logarithmic titre ( $\log_{10}$ TCID<sub>50</sub>/ml) as described above.

## 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. These mixtures or PBS as control were 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).

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## 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:

- The titre of the test virus suspension allowed the determination of a  $\geq 4 \log_{10}$  reduction (maximal virus reduction  $\geq 4.02 \pm 0.31$ , LVP)
- The test product (1.0 %) showed cytotoxicity in the 1:100 dilutions thus allowing the detection of a  $4 \log_{10}$  reduction of virus titre.
- The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *BHK 21-cells* showed no significant difference ( $< 1 \log_{10}$ ; EN 5.7) of virus titre:  $6.63 \pm 0.49$  (PBS, LVP) versus  $6.13 \pm 0.37$  (1:5,000 dilution of disinfectant as 1.0 % solution, LVP)  $\log_{10}$  TCID<sub>50</sub>/ml.
- The control of efficacy for suppression of disinfectant's activity (1.0 %) showed no decrease ( $\leq 0.5 \log_{10}$ ; EN 5.5.5.1) in virus titre ( $6.25 \pm 0.48$  versus  $6.50 \pm 0.46 \log_{10}$  TCID<sub>50</sub>/ml).
- 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.

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## 7. Results

Results of examination are shown in tables 1 to 13. Tables 1 to 11 demonstrate the raw data, whereas tables 12 (a+b) and 13 give a summary of results.

Due to the high cytotoxicity of the test product, only the 0.1 % solution (demonstration of non-active range) was tested using the end point dilution method. The test product as 0.1 % solution was not active within 30 minutes of exposure time (table 2).

In parallel to the end point dilution method the large volume plating method (LVP) was introduced testing the test product as 1.0 % and 0.5 % solutions with 5 and 15 minutes of exposure time. In a first assay the virus titres in the twofold assay were  $\log_{10} \text{TCID}_{50}/\text{ml} = 6.50 \pm 0.46$  and  $6.63 \pm 0.43$  (table 6). The mean value was  $6.56 \pm 0.31$ . In a second assay the virus titres in the twofold assay were  $\log_{10} \text{TCID}_{50}/\text{ml} = 6.50 \pm 0.46$  and  $6.63 \pm 0.41$  (table 10). The mean value was  $6.56 \pm 0.31$ .

The test product as 1.0 % solution was active after 5 minutes of exposure time (tested in duplicate on two different days, tables 7 and 11). No residual virus was found in 576 cell culture units in both assays. The result according to the formula of Poisson was  $\leq 2.54 \log_{10} \text{TCID}_{50}$ . The reduction factor was therefore  $\geq 4.02 \pm 0.31$  ( $6.56 \pm 0.31 \log_{10} \text{TCID}_{50}$  minus  $\leq 2.54 \log_{10} \text{TCID}_{50}$ ) in the first assay and  $\geq 4.02 \pm 0.31$  ( $6.56 \pm 0.31 \log_{10} \text{TCID}_{50}$  minus  $\leq 2.54 \log_{10} \text{TCID}_{50}$ ) after 5 minutes of exposure time in the second assay. The mean value was  $\geq 4.02 \pm 0.22$ . This corresponded to an inactivation of  $\geq 99.99$  %.

The test product as 0.5 % solution was not active within 15 minutes of exposure time (table 9). Residual virus was found in 111 of 576 cell culture units. The result according to the formula of Taylor was  $4.16 \log_{10} \text{TCID}_{50}$ . The reduction factor was therefore  $2.40 \pm 0.31$  ( $6.56 \pm 0.31 \log_{10} \text{TCID}_{50}$  minus  $4.16 \log_{10} \text{TCID}_{50}$ ) after 15 minutes of exposure time.

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## 8. Conclusion

The surface disinfectant Hexaquart XL tested as 1.0 % solution demonstrated activity against MVA after an exposure time of 5 minutes under dirty conditions.

Therefore, the surface disinfectant Hexaquart XL can be declared as active against MVA as follows:

**1.0 %      5 minutes      dirty conditions**

**Bremen, 26/04/2017**

**- Dr. Britta Becker -**  
Head of Laboratory

**- Dr. Dajana Paulmann -**  
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## 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 (BGBl. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBl. 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.

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The test results in this test report relate only to the items examined.

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## 11. Literature

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## Appendix:

### Legend to the Tables

Table 1:	Raw data for Hexaquart XL (0.1 %) tested against MVA (1 <sup>st</sup> assay)
Table 2:	Raw data for Hexaquart XL (0.1 %) tested against MVA (2 <sup>nd</sup> assay)
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 (1.0 %)
Table 5:	Raw data (MVA) for cell sensitivity (1.0 %) (LVP)
Table 6:	Determination of virus titre (LVP) (1 <sup>st</sup> assay)
Table 7:	Inactivation of MVA by Hexaquart XL (1.0 %) (5 minutes) (LVP)
Table 8:	Inactivation of MVA by Hexaquart XL (0.5 %) (5 minutes) (LVP)
Table 9:	Inactivation of MVA by Hexaquart XL (0.5 %) (15 minutes) (LVP)
Table 10:	Determination of virus titre (LVP) (2 <sup>nd</sup> assay)
Table 11:	Inactivation of MVA by Hexaquart XL (1.0 %) (5 minutes) (LVP)
Table 12 (a+b):	Summary of results (end point dilution method) with Hexaquart XL and MVA
Table 13:	Summary of results (LVP, 1:5,000) with Hexaquart XL and MVA

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## Legend to the Figures

Figure 1:                      Virus-inactivating properties of Hexaquart XL (1.0 %) (LVP)

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

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**Table 1: Raw data for Hexaquart XL (0.1 %) tested against MVA at 20 °C (quantal test; 8 wells) (#4930) (1<sup>st</sup> assay)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
test product	0.1 %	dirty conditions	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	tttt tttt	4444 4444	4444 4444	4444 4444	4003 3033	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	4444 4444	4444 4444	3444 4444	3414 0300	0004 0000	0000 0000	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	0.1 %	dirty conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	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	2323 0401	0004 0003	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)

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**Table 2: Raw data for Hexaquart XL (0.1 %) tested against MVA at 20 °C (quantal test; 8 wells) (#4945) (2<sup>nd</sup> assay)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
test product	0.1 %	dirty conditions	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	4443 4444	0403 4200	0000 0000	0000 0000	n.d. n.d.	n.d. n.d.
test product cytotoxicity	0.1 %	dirty conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	3232 3032	0000 0000	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4344	0440 4244	0030 0400	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)

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**Table 3: Raw data for formaldehyde solution (0.7 %) tested against MVA at 20 °C (quantal test; 8 wells) (#4945)**

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log <sub>10</sub> )								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7 % (m/V)	PBS	5	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	0000 0000	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	0000 0000	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	4432 0400	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)

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**Table 4: Raw data for control of efficacy for suppression of disinfectant's activity (1.0 %) (#4945)**

Product	Interfering substance	dilutions (log <sub>10</sub> )								
		1	2	3	4	5	6	7	8	9
test product	dirty conditions	tttt tttt	tttt tttt	4444 4444	2324 2230	4432 0022	0000 0100	0000 0000	0000 0000	n.d.
corresponding virus control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	0440 4244	0030 0400	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)

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**Table 5: Raw data (MVA) for cell sensitivity (1.0 % solution) (#4945) (LVP)**

Product	Dilution	Dilutions (log <sub>10</sub> )								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	2433 0204	0200 1002	0000 0000	0000 0000	n.d.
test product	1:5,000	4444 4444	4444 4444	4444 4444	4444 4444	2230 4400	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)

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**Table 6: Determination of virus titre (LVP) at 20 °C (#4930) (1<sup>st</sup> assay)**

Virus titration	Interfering substance	dilutions (log <sub>10</sub> )								
		1	2	3	4	5	6	7	8	9
1 <sup>st</sup> control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	2323 0401	0004 0003	0000 0000	0000 0000	n.d.
2 <sup>nd</sup> control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	3232 4033	0000 0003	0003 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)

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**Table 7: Inactivation of MVA by Hexaquart XL (1.0 %) at 20 °C (5 minutes) (LVP, 1:5,000) (#4930) (1<sup>st</sup> assay)**

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	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)

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**Table 8: Inactivation of MVA by Hexaquart XL (0.5 %) at 20 °C (5 minutes) (LVP, 1:5,000) (#4930)**

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	plate 1/6	4330 3033	4004 3202	0042 4444	3400 4032	4033 2433	0034 0043	2304 0442	3234 3040	0441 2303	0020 3330	0004 4012	4033 0434
	plate 2/6	4014 0000	4200 0304	4430 0442	3004 0000	0030 0233	2330 2403	0443 0003	2210 4340	0003 3343	4304 4030	0433 2340	2332 4332
	plate 3/6	4040 2443	3300 4223	4442 0244	4003 3043	2023 3030	2334 0403	2233 0434	3304 3433	0023 0044	4332 2043	0300 0444	3200 2443
	plate 4/6	4324 0200	3024 2304	4344 3444	4024 0444	3343 3343	4333 2223	3023 3023	2030 3034	4244 0430	3400 3040	0032 4343	3304 3430
	plate 5/6	2303 0433	4440 3430	0300 4403	1043 4040	3322 4440	4044 0034	0304 3433	2340 3042	2003 3340	2043 4404	2034 0222	3004 3333
	plate 6/6	0322 2200	4300 3034	0334 4023	3423 3300	3023 4344	0303 0314	0333 3023	3334 4103	4043 3323	3000 3404	3340 3033	4034 4304

t = cytotoxic

0 = no virus detectable

1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

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**Table 9: Inactivation of MVA by Hexaquart XL (0.5 %) at 20 °C (15 minutes) (LVP, 1:5,000) (#4930)**

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	plate 1/6	3200 0300	0100 3000	0003 3000	0000 0000	0000 0000	0000 4000	0040 2040	0204 3044	0000 0400	0004 0000	0002 0000	3000 0020
	plate 2/6	0000 0003	0400 0000	0004 4000	0400 2002	0023 0400	0000 0004	0430 0200	2000 0030	0000 0000	0440 0202	0001 0044	0003 0000
	plate 3/6	0004 0000	0044 0400	0000 0000	0020 0000	0000 0000	0000 0000	0000 3300	0000 0000	0010 0000	0000 0000	0400 0030	0002 4000
	plate 4/6	3432 0020	0000 4000	0003 0003	1002 0300	0000 3003	0020 0300	3000 0000	0002 0300	0200 0000	0000 4000	4000 0030	0400 0400
	plate 5/6	4000 0000	0400 0000	0040 0000	0040 0000	0000 0020	0000 0000	0030 0000	0040 0440	0400 0000	0003 0000	0002 0020	0030 0020
	plate 6/6	0003 0044	4000 0000	0000 0040	0400 0000	0000 0000	0004 0300	0000 0000	0000 0000	0000 0000	0000 0000	0040 0040	0000 0003

t = cytotoxic

0 = no virus detectable

1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

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**Table 10: Determination of virus titre (LVP) at 20 °C (#4945) (2<sup>nd</sup> assay)**

Virus titration	Interfering substance	dilutions (log <sub>10</sub> )								
		1	2	3	4	5	6	7	8	9
1 <sup>st</sup> control	dirty conditions	4444	4444	4444	4444	0440	0030	0000	0000	0000
		4444	4444	4444	4344	4244	0400	0000	0000	0000
2 <sup>nd</sup> control	dirty conditions	4444	4444	4444	4444	0443	0020	0000	0000	0000
		4444	4444	4444	4444	2443	0300	0000	0000	0000

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)

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**Table 11: Inactivation of MVA by Hexaquart XL (1.0 %) at 20 °C (5 minutes) (LVP, 1:5,000) (#4945)**

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	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)

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**Table 12a: Summary of results (end point dilution method) with Hexaquart XL and MVA**

Product*	Con- centration	Interfering substance	Level of cytotoxicity	log <sub>10</sub> TCID <sub>50</sub> /ml after ....min					> 4 log <sub>10</sub> reduction after ...min
				1	5	15	30	60	
test product (1)	0.1 %	dirty conditions	3.50	n.d.	6.13±0.37	6.25±0.44	n.d.	n.d.	> 15 (RF = 0.25±0.64)
test product (2)	0.1 %	dirty conditions	2.50	n.d.	n.d.	n.d.	6.00±0.38	n.d.	> 30 (RF = 0.50±0.60)

\*The number in brackets gives the number of the corresponding virus control (see table 12b)

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

**Table 12b: Summary of results (end point dilution method) with Hexaquart XL and MVA**

Product	Con- centration	Interfering substance	Level of cytotoxicity	log <sub>10</sub> TCID <sub>50</sub> /ml after ....min					> 4 log <sub>10</sub> reduction after ... min
				0	5	15	30	60	
formaldehyde	0.7 % (w/v)	PBS	3.50	n.d.	≤ 3.50±0.00	≤ 3.50±0.00	≤ 3.50±0.00	≤ 3.50±0.00	≥ 5 (RF ≥ 2.63±0.26)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	6.13±0.37	n.a.
virus control (1)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.50±0.46	n.a.
virus control (2) +suppression	n.a.	dirty conditions	n.a.	6.38±0.25	n.d.	n.d.	n.d.	6.50±0.46	n.a.
suppression control	1.0 %	dirty conditions	3.50	n.d.	n.d.	n.d.	6.25±0.48	n.d.	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.
sens. product	1.0 % → 1:1,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.

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

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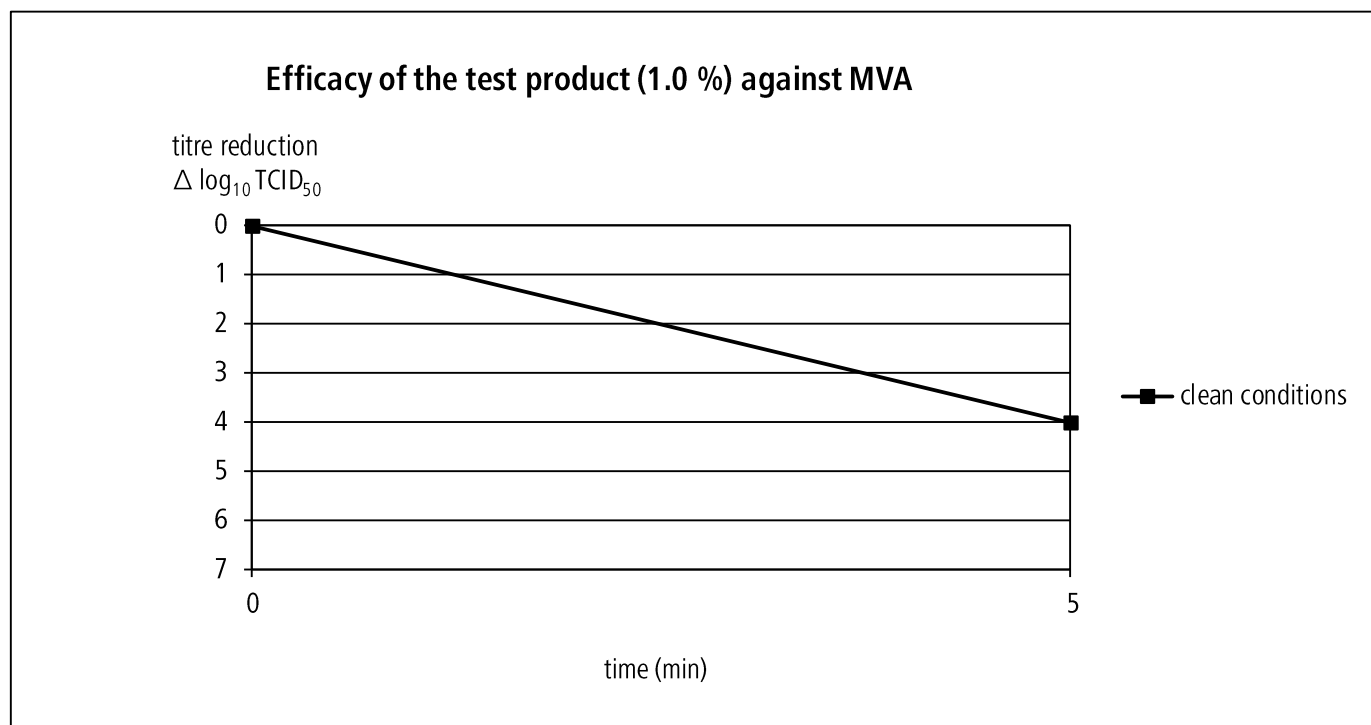
**Table 13: Summary of results (LVP, 1:5,000) with Hexaquart XL and MVA**

Product*	Con- centration	Interfering substance	Level of cytotoxicity	log <sub>10</sub> TCID <sub>50</sub> /ml after ....min					> 4 log <sub>10</sub> reduction after ...min
				1	5	15	30	60	
test product (1)	1.0 %	dirty conditions	n.a.	n.d.	≤ 2.54	n.d.	n.d.	n.d.	5 (RF ≥ 4.02±0.31)
test product (2)	1.0 %	dirty conditions	n.a.	n.d.	≤ 2.54	n.d.	n.d.	n.d.	5 (RF ≥ 4.02±0.31)
test product (1)	0.5 %	dirty conditions	n.a.	n.d.	4.92	4.16	n.d.	n.d.	> 15 (RF = 2.40±0.31)
virus control (1)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.50±0.48 6.63±0.43 (Ø6.56±0.31)	n.a.
virus control (2)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.50±0.46 6.63±0.41 (Ø6.56±0.3)	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.49	n.a.
sens. product	5.0 % → 1:1,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.13±0.37	n.a.

\*The number in brackets gives the number of the corresponding virus control

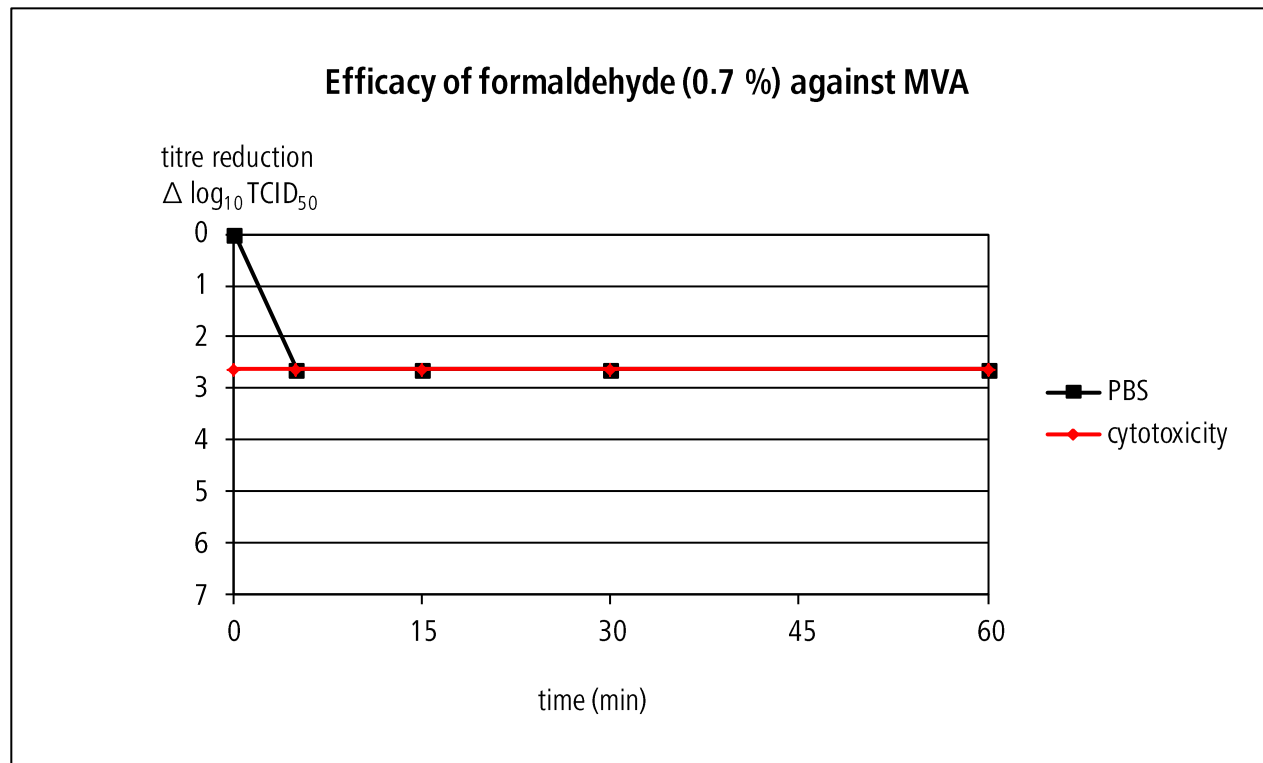
n.a. = not applicable    n.d. = not done    sens. = sensitivity    n.c. = not calculable

**Figure 1: Virus-inactivating properties of Hexaquart XL (1.0 %)**



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



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