EN ISO/IEC 17025
L236

EVS-EN 17111:2018
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Quantitative carrier test for the evaluation of virucidal activity in the medical area (phase 2, step 2)

Test report no 763

1. **General information**

Client:	Medisept Sp. z o.o., ul. Ludwika Spiessa 4, 20 270 Lublin, Poland
Date of order:	2022/11/29

2. **Identification of sample**

Name of the product:	VIRUTON PULVER

Microbiologist
Ljudmila Shljapnikova, ph.D

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EESTI VABARIIK
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Head of the Laboratory

Batch number:	220902_4
Aplication	Solid product for disinfection of medical instruments
Manufacturer:	Medisept Sp. z o.o
Date of delivery:	2022/11/21
Storage conditions:	room temperature and darkness
Apperance of the product:	Dry blend of white and blue granules and powder
Active substance and concentration	44 % Sodium percarbonate, 26 % TAED

3. **Test conditions**

Test period:	2022/12/05 – 2022/12/27
End of testing	2022/12/27
Product test concentrations:	0,02%, 0,5%, 1,0%, 2,0%
Exposure time:	10 min.
Test temperature:	19,5°C ± 0,5°C
Interfering substance:	for dirty conditions (bovine albumine 3,0 g/l and sheep erythrocytes 3 ml/l)
Neutralization of active substance:	Immediate dilution, 30 min, +4°C
Diluent	PBS, EMEM + 2% FBS
Stability of product in the mix with virus and interfering substance	Flocculation forms with interfering substance
Test organisms:	Vaccinia virus ATCC-VR-1508 strain MVA

4. Materials

1.1 Culture medium and reagents

- Eagle´s Minimum Essential Medium (EMEM, Corning, REF 10-009-CVR)
- Dulbecco´s modification of Eagle´s Medium (DMEM, Corning, REF 10-014-CVR)
- Fetal Bovine Serum (FBS, Corning, REF 35-016-CV)
- 50% % Glutaraldehyde solution (Thermo Scientific, A10500)

- Aqua bi-distillate (Interflo OU, Estonia)
- PBS (Phosphate Buffered Saline) (Interflo OU, Estonia)
- BSA (Bovine Serum Albumin fr.V) (Roche Diagnostic GMBH)
- ShE (Defibrinated sheep erythrocytes) (BioTRADING Benelux B.V.)

1.2 Virus and cells

Strain: Modified Vaccinia virus Ankara (MVA), ATCC-VR-1508, LOT 5016818 (LGC Standards GmbH, Wesel, Germany)
BHK-21 cells (C-13), ATCC-CCL-10, LOT 63226279, Kidney, Syrian Hamster (Mesocricetus auratus). Morphology – fibroblast-like (LGC Standards GmbH, Wesel, Germany)

1.3 Apparatus, glassware and small items of equipment

- CO2 incubator Memmert GmbH & Co. KG, ICO50 med, no D218.1233, Germany
- Agitator Vortex V-3, no 7EE0011 Biosan SIA, Latvia
- Digital pH-meter, Instrument: HI2002, SN:CO646088, Hanna, USA
- Centrifuge Centric 322A, Code: 464.000, Domel, d.o.o. BU Laboratory system, Slovenia
- Inverted microscope Motic AE2000 Series, Motic China Group, Ltd, China
- Water bath WB/OB 7-45 WBU 45 Memmert GmbH & Co. KG, Germany
- Adjustable and fixed-volume pipettes, Sartorius Lab Instruments GmbH & Co. KG Germany
- Tissue Culture Plate, 96-well, Falcon, REF 353072 Corning Incorporated, USA
- Tissue Culture flask Falcon pour culture cellulaire, REF 353108 Corning Incorporated, USA
- Tissue Culture flask Falcon pour culture cellulaire, REF 353136 Corning Incorporated, USA
- 50 mL Polypropylene Conical Tube Falcon a Corning Brand, REF 352070, Corning Science Mexico S.A. de C.V., Mexico
- Frosted Glass Carriers 15mm x60mm x1mm, one surface sandblasted, Interflo OU, Estonia

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5. Methods

5.1 Preparation of test virus suspension

For the preparation of the test virus suspension according to EN 5.4.1 BHK-21 cells (C-13), ATCC-CCL-10 which were cultivated with Eagle's Minimum Essential Medium (EMEM) supplemented with L-glutamine, sodium pyruvate and 10 % fetal calf serum (FCS), were infected with a multiplicity of infection of 0,1 at 37°C. After cells showed a cytopathic effect, they were subjected to a threefold freeze/thaw procedure followed by a low speed centrifugation in order to sediment cell debris. After aliquotation of the supernatant, test virus suspension was stored at –80°C.

5.2 Preparation of disinfectant (dilutions)

The test product was evaluated as 100 % -+solution. Due to the addition of the fresh prepared hard water (5.2.2.7) heated to 35°C ± 5,0°C and mix during the 20 min. (5 min. for dilution and 15 min for product activation) were prepared solutions for test. The test product was evaluated as 2,0 %, 1,0%, 0,5% solution and 0,02% solutions (demonstrations of non –active range).

5.3 Inoculation of the carriers

Glass carriers were placed in Petri dishes in horizontal position. Pipette 0,05ml of the mixture of test suspension and interfering substance on the inoculation square of the carrier. Inoculum were dried under an air laminar flow at 20°C in 15 min.

5.4 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 microtiter plate to 0.1 ml of freshly trypsinised cells (10^3 – 10^4 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 after seven days. Calculation of the infective dose $TCID_{50}$ /ml was calculated with the method of Spearman (2) and Kärber 3) with the following formula:

$$-\log_{10} TCID_{50} = Xo - 0{,}5 + \sum r/n$$

meaning
Xo = log10 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.

5.5 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titer in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).
According to the EN 17111, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by 4 log10 steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99,99 %.

5.6 Inactivation assay

Determination of virucidal activity has been carried out in accordance to EN 5.5. The test product was examined as 2,0 % 1,0%, 0,5% solutions and as 0,02% solution (demonstrations of non –active range) in water at 20°C according to EN 17111, 10 min. were chosen as contact times.

Immediately at the end of the chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8} at +4°C.

Titrations of the virus control were performed after the longest exposure time (EN 5.5.6).
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.

5.7 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.3.1.

5.8 Cell sensitivity to virus

For the control of cell sensitivity to virus 0.3 parts by volume of water were mixed with 9.7 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 5.5.3.2b).
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 performed according (EN 5.5.4).

5.10 Reference virus inactivation test

As reference for the test validation 50 % glutaraldehyde solution according to EN 5.5.5 was included. 5 min was chosen as contact time. In addition, cytotoxicity of glutaraldehyde test solution was determined following EN 5.5.3.1.

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 ≥6,13±0,00).
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 5.5.5) was ≥7,00 (≥4,0 log$_{10}$) after 5 min.
c) The test product (2,0 %) showed cytotoxicity in the 1:10 dilutions thus allowing the detection of a 4 log $_{10}$ reduction of virus titre
d) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) BGM cells showed no significant difference <1 log $_{10}$ of virus titre: 7,75 (PBS) versus 7,50 (1:1000 dilutions of disinfectant) log 10 TCID$_{50}$/ml.

e) The control of efficacy for suppression of disinfectant`s activity (0,5%) showed no decrease in virus titer (8,50 versus 8,13 $\log_{10}$ $TCDI_{50}$/ml).

Since all criteria according EN 5.7 fulfilled, examination with vaccinia virus according to EN 17111 is valid.

7. Results

Results for examination are shown in tables 1 to 7. Tables 1 to 6 demonstrate the raw data, whereas table 7 gives a summary of results.

The diluted test product (2,0%, 1,0% assay) was able to inactivate vaccinia virus after 10 min. in this quantitative carrier test (Table 1,2). The reduction factor was ≥6,13±0,00 at this time point. This corresponded to in inactivation of ≥ 99.99 %.
The test product in 0,5 % assay was also able to inactivate vaccinia virus after 10 min. in this quantitative suspension test (Table 3). The reduction factor was ≥6,13±0,00 at this time point.

8. Conclusion

The disinfectant for medical instruments VIRUTON PULVER tested with concentrations 2,0%, 1,0%, 0,5 % demonstrated effectiveness against Vaccinia virus after an exposure time of 10 min. under dirty conditions.
Therefore, the disinfectant for medical instruments VIRUTON PULVER (LOT 220902_4) can be declared as active against Vaccinia virus ATCC-VR-1508, thereby showing virucidal activity against enveloped viruses presented in Annex B_ EN17111: 2018 guideline as follows.

0,5% 10 MIN

Tallinn, 2022/12/28

Dr. Ljudmila Šljapnikova

9. Reference

1. EN 17111:2018: Chemical disinfectants and antiseptics – Quantitative carrier test for the evaluation of virucidal activity for instruments used in the medical area – Test method and requirements (phase 2, step 2)

Annex:

Legend to the tables

Table 1: Raw data for VIRUTON PULVER (2,0%) tested against Vaccinia virus

Table 2: Raw data for VIRUTON PULVER (1,0%) tested against Vaccinia virus

Table 3: Raw data for VIRUTON PULVER (0,5%) tested against Vaccinia virus

Table 4: Raw data for VIRUTON PULVER (0,02%) tested against Vaccinia virus

Table 5: Raw data for GLUTARLDEHYDE solution (50 %) tested against Vaccinia virus

Table 7: Raw data for control of efficacy for suppression of disinfectant's activity (0,5 %)

Table 8: Raw data (Vaccinia virus) for cell sensitivity (0,5 %)

Table 9: Summary of results with VIRUTON PULVER and Vaccinia virus (a)

Legend to the Figures

Figure 1: Virus-inactivating properties of VIRUTON PULVER + ref. Glutaraldehyde (50 %)

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Table 1: Raw data for VIRUTON PULVER (2,0 %) tested against Vaccinia virus at 20 °C (quantal test; 8 wells)

Product	Concentration	Interfering substance	Contact time	Dilution range (log 10)							
				1	2	3	4	5	6	7	8
Test product	2,0%	3,0 g/L BSA + 3,0 ml/L ShE	10 min	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Test product cytotoxicity	2,0 %	3,0 g/L BSA + 3,0 ml/L ShE	n. a.	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 + 3,0 ml/L ShE	0 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3332 2223
Water control	n. a.	3,0 g/L BSA + 3,0 ml/L ShE	10 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2222 2332	0000 0200

n. a. – not applicable
n.d. – not done
0 – no virus present
T – cytotoxic
1 to 4 – virus present (degree of CPE in 8 cells units) (wells of microtiter plates)

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Table 2: Raw data for VIRUTON PULVER (1,0 %) tested against Vaccinia virus at 20 °C (quantal test; 8 wells)

Product	Concentration	Interfering substance	Contact time	Dilution range (log 10)							
				1	2	3	4	5	6	7	8
Test product	1,0%	3,0 g/L BSA + 3,0 ml/L ShE	10 min	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Test product cytotoxicity	1,0 %	3,0 g/L BSA + 3,0 ml/L ShE	n. a.	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 + 3,0 ml/L ShE	0 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3332 2223
Water control	n. a.	3,0 g/L BSA + 3,0 ml/L ShE	10 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2222 2332	0000 0200

n. a. – not applicable
n.d. – not done
0 – no virus present
t – cytotoxic
1 to 4 – virus present (degree of CPE in 8 cells units) (wells of microtiter plates)

Table 3: Raw data for VIRUTON PULVER (0,5 %) tested against Vaccinia virus at 20 °C (quantal test; 8 wells)

Product	Concentration	Interfering substance	Contact time	Dilution range (log 10)							
				1	2	3	4	5	6	7	8
Test product	0,5%	3,0 g/L BSA + 3,0 ml/L ShE	10 min	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Test product cytotoxicity	0,5 %	3,0 g/L BSA + 3,0 ml/L ShE	n. a.	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 + 3,0 ml/L ShE	0 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3332 2223
Water control	n. a.	3,0 g/L BSA + 3,0 ml/L ShE	10 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2222 2332	0000 0200

n. a. – not applicable
n.d. – not done
0 – no virus present
T – cytotoxic
1 to 4 – virus present (degree of CPE in 8 cells units) (wells of microtiter plates)

Table 4: Raw data for VIRUTON PULVER (0,02 %) tested against Vaccinia virus at 20 °C (quantal test; 8 wells)

Product	Concentration	Interfering substance	Contact time	Dilution range (log 10)							
				1	2	3	4	5	6	7	8
Test product	0,02%	3,0 g/L BSA + 3,0 ml/L ShE	10 min	tttt tttt	4444 4444	4444 4444	2222 2220	0000 0000	0000 0000	0000 0000	0000 0000
Test product cytotoxicity	0,02 %	3,0 g/L BSA + 3,0 ml/L ShE	n. a.	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 + 3,0 ml/L ShE	0 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3332 2223
Water control	n. a.	3,0 g/L BSA + 3,0 ml/L ShE	10 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2222 2332	0000 0200

n. a. – not applicable
n.d. – not done
0 – no virus present
T – cytotoxic
1 to 4 – virus present (degree of CPE in 8 cells units) (wells of microtiter plates)

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Table 5: Raw data for Glutaraldehyde solution (50,0 %) tested against Vaccinia virus at 20 °C (quantal test; 8 wells)

Product	Concentration	Interfering substance	Contact time	Dilution range (log 10)							
				1	2	3	4	5	6	7	8
Glutaraldehyde	50,0 % (m/V)	PBS	5 min	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
Glutaraldehyde cytotoxicity	50,0 % (m/V)	PBS	n. a.	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.	n.d.
Virus control	n. a.	PBS	5 min	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2332 2323

n. a. – not applicable
n.d. – not done
0 – no virus present
T – cytotoxic
1 to 4 – virus present (degree of CPE in 8 cells units) (wells of microtiter plates)

Table 6: Raw data for control efficacy for suppression of disinfectant activity 0,5 %,

Product	Concentration	Interfering substance	Dilution range (log 10)							
			1	2	3	4	5	6	7	8
VIRUTON PULVER	0,5 %	3,0 g/L BSA + 3,0 ml/L ShE	tttt tttt	4444 4444	4444 4444	3344 4444	3333 3333	3223 3222	2222 2222	2200 0222
Virus control	n. a.	3,0 g/L BSA + 3,0 ml/L ShE	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2222 2222

n. a. – not applicable
n.d. – not done
0 – no virus present
t – cytotoxic
1 to 4 – virus present (degree of CPE in 8 cells units) (wells of microtiter plates)

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Table 7: Raw data (vaccinia virus) for cell sensitivity (0,5%)

Product	Dilution	Interfering substance	Dilution range (log 10)							
			1	2	3	4	5	6	7	8
PBS	-	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2333 2333
VIRUTON PULVER	1:1000	3,0 g/L BSA + 3,0 ml/L ShE	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	2222 2222

n. a. – not applicable
n.d. – not done
0 – no virus present
t – cytotoxic
1 to 4 – virus present (degree of CPE in 8 cells units) (wells of microtiter plates)

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Table 8: Summary of results of activity of the product VIRUTON PULVER with Vaccinia virus, Ankara, MVA, ATCC VR – 1508

Assay	Interfering substance	Concentration	Cytotoxicity level log_{10}	log_{10} TCID$_{50}$ / ml after …					Reduction with the confidence interval of 95 %
				0 min	5 min	10 min	30 min	60 min	
Test product	3,0 g/L BSA + 3,0 ml/L ShE	2,0 %	1,5			≤1,50			≥6,13±0,00
		1,0 %	1,5			≤1,50			≥6,13±0,00
		0,5 %	1,5			≤1,50			≥6,13±0,00
		0,02 %	1,5			4,38±0,13			3,25±0,25
Water control	3,0 g/L BSA + 3,0 ml/L ShE	0%	n.a.			7,63±0,13			
Virus control	3,0 g/L BSA + 3,0 ml/L ShE	n.a.	n.a.	8,50±0,00			8,50		n.a.
Glutaraldehyde	PBS	50 % (w:v)	1,5		≤1,50				≥7,00
Virus control for Glutaraldehyde	PBS	n.a.	n.a.		8,50				n.a.
Suppression of disinfectant activity	3,0 g/L BSA + 3,0 ml/L ShE	0,5 %	1,5				8,13		n.a.
Cell sensitivity control PBS	PBS	n.a.	n.a.					7,75	n.a.
Cell sensitivity control test product	3,0 g/L BSA + 3,0 ml/L ShE	1:1000	n.a.					7,50	n.a.

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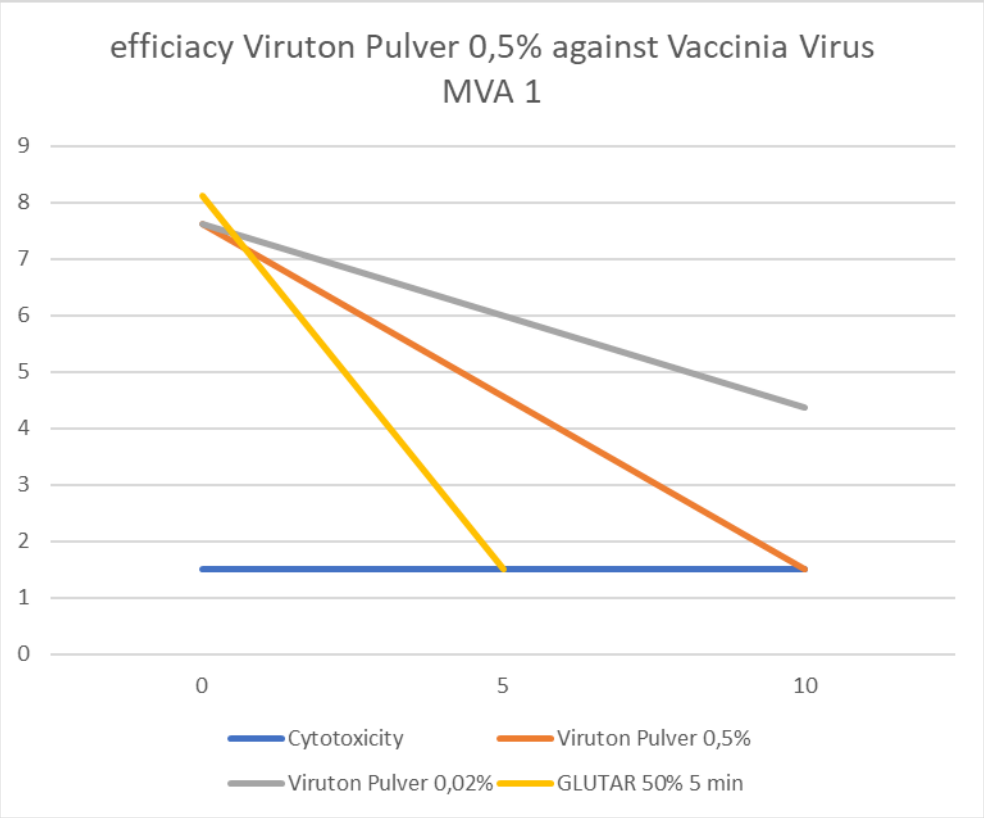
Figure 1



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