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Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute according to ČSN EN ISO/IEC 17025.

Copy No.: 1
Issue No.: 1

Test report No. D330/2016

DETERMINATION OF VIRUCIDAL (EN 14476+A1) ACTIVITY OF THE PRODUCT **VIRUTON PULVER**

Sample ID: D330/2016
Sample name: **Viruton Pulver**
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Producer: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland

Incoming date:
28.11.2016

Delivery date:
4.4.2017

Hodonín, 4.4.2017

Ing. Jana Šlitrová, Head of Laboratory

Digitally signed by Palii Nina
Date: 2023.01.05 11:10:18 EET
Reason: MoldSign Signature
Location: Moldova

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D330/2016
Rep No: 34
Sample name: **Viruton Pulver**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Batch No: 20160819_55

Sampling date: 22.11.2016
Sample delivered: 28.11.2016
Testing date: 24.2. – 31.3.2017
Delivered amount: 4 x 20 g

Subject of testing:
Determination of virucidal activity of the product.

Identification of the sample:

Name of the product:	**Viruton Pulver**
Batch number:	20160819_55
Date of manufacture:	2016.08.19
Expiry date:	2017.08.19
Manufacturer:	Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Incoming date:	28.11.2016
Storage conditions:	stated by the manufacturer

Active ingredients in 100 g:
44g Sodium percarbonate (CAS: 15630-89-4), 26g TEAD (CAS: 10543-57-4)

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A1:2015)
Period of analysis:	24.2. – 6.3.2016
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Product diluent:	hard water
Appearance of the product:	white powder with blue particles
Test concentration:	1% and 2%
Contact time:	10 and 30 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K47740803613, expiry date: 31.3.2018
Test virus:	*Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 (6^{th} passage)
Cell lines:	HeLa cells
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 96 hours and additional period of 48 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method.

Preparation of the test

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of the cell culture
3. Preparation of the test virus suspension
4. Test of the viral infectivity
5. Virus titration with the interfering substance
6. Cytotoxicity of the product
7. Reference virus inactivation test
8. Test procedure for the virucidal activity of the product

Note:
Virucidal activity – the capability of a product to produce a reduction in the number of infectious virus particles under defined conditions by at least 4 (lg) orders.

The standard:
EN 14476:2013 +A1:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1) August 2013 + September 2015

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D330/2016
Rep No: 34
Sample name: **Viruton Pulver**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Batch No: 20160819_55

Sampling date: 22.11.2016
Sample delivered: 28.11.2016
Testing date: 24.2. – 31.3.2017
Delivered amount: 4 x 20 g

The Number of CFU in the tested product **Viruton Pulver**: $<10^1$ CFU/g

1. Testing the efficacy of chemical disinfectant **Viruton Pulver** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Tab No. 1.1 Table of results of product **Viruton Pulver** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 10 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
Viruton Pulver	2%	clean	-	4.17	-	-
Viruton Pulver	2%	dirty	3.50	4.50	-	-
Viruton Pulver	1%	clean	-	-	3.50	-
Viruton Pulver	1%	dirty	-	-	3.83	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	5.83	5.50
			Virus titration, time = 0			
Virus control	-	PBS	9.00	-	9.17	9.17
Virus control	-	clean	9.00	9.00	9.17	-
Virus control	-	dirty	9.00	9.00	9.17	-

Tab No. 1.2 Testing the efficacy of chemical disinfectant **Viruton Pulver** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
2%	9.00	clean	10 min	4.17	**4.83**
2%	9.00	dirty	10 min	4.50	**4.50**
1%	9.00	clean	30 min	3.50	**5.50**
1%	9.00	dirty	30 min	3.83	**5.17**

2. Evaluation of virucidal activity of the product **Viruton Pulver**

Tab No. 2.1 The efficacy of chemical disinfectant **Viruton Pulver** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013+A1:2015)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013+ A1:2015	$\Delta\log_{10}$ $TCID_{50}$
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	10	2	clean	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	10	2	dirty	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	30	1	clean	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	30	1	dirty	≥ 4	**> 4**

Note:
$TCID_{50}$- 50% infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE in 50% of cell culture units

Prepared by: Bc. Iva Čížová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D330/2016
Rep No: 34
Sample name: **Viruton Pulver**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Batch No: 20160819_55

Sampling date: 22.11.2016
Sample delivered: 28.11.2016
Testing date: 24.2. – 31.3.2017
Delivered amount: 4 x 20 g

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A1:2015)
Period of analysis:	24.3. – 31.3.2017
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Product diluent:	hard water
Appearance of the product:	white powder with blue particles
Test concentration:	1% and 2%
Contact time:	10 and 30 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K47740803613, expiry date: 31.3.2018
Test virus:	*Murine norovirus (MNV)* strain S99, RVB-651 (2nd passage)
Cell lines:	RAW 264.7 *Murine macrophage* cell line
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 72 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method.

Preparation of the test

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of cell culture
3. Preparation of the test virus suspension
4. Test of viral infectivity
5. Virus titration with interfering substance
6. Cytotoxicity of the product
7. Reference virus inactivation test
8. Test procedure for virucidal activity of product

Note:
Virucidal activity – the capability of a product to produce a reduction in the number of infectious virus particles under defined conditions by at least 4 (lg) orders.

The standard:
EN 14476:2013 +A1:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1) August 2013 + September 2015

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D330/2016
Rep No: 34
Sample name: **Viruton Pulver**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Batch No: 20160819_55

Sampling date: 22.11.2016
Sample delivered: 28.11.2016
Testing date: 24.2. – 31.3.2017
Delivered amount: 4 x 20 g

3. Testing the efficacy of chemical disinfectant **Viruton Pulver** on *Murine norovirus (MNV)* strain S99, RVB-651

Tab No. 3.1 Table of results of product **Viruton Pulver** on *Murine norovirus (MNV)* strain S99, RVB-6515

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 10 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
Viruton Pulver	2%	clean	-	4.17	-	-
Viruton Pulver	2%	dirty	3.50	4.50	-	-
Viruton Pulver	1%	clean	-	-	4.17	-
Viruton Pulver	1%	dirty	-	-	3.83	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	7.17	5.83
			Virus titration, time = 0			
Virus control	-	PBS	9.50	-	9.50	9.67
Virus control	-	clean	9.50	9.50	9.67	-
Virus control	-	dirty	9.50	9.50	9.67	-

Tab No. 3.2 Testing the efficacy of chemical disinfectant **Viruton Pulver** on *Murine norovirus (MNV)* strain S99, RVB-651

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
2%	9.50	clean	10 min	4.17	**5.33**
2%	9.50	dirty	10 min	4.50	**5.00**
1%	9.50	clean	30 min	4.17	**5.33**
1%	9.50	dirty	30 min	3.83	**5.67**

4. Evaluation of virucidal activity of the product **Viruton Pulver**

Tab No. 4.1 The efficacy of chemical disinfectant **Viruton Pulver** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013+A1:2015)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013+ A1:2015	$\Delta\log_{10}$ $TCID_{50}$
Murine norovirus (MNV) strain S99, RVB-651	20	10	2	clean	≥ 4	**> 4**
Murine norovirus (MNV) strain S99, RVB-651	20	10	2	dirty	≥ 4	**> 4**
Murine norovirus (MNV) strain S99, RVB-651	20	30	1	clean	≥ 4	**> 4**
Murine norovirus (MNV) strain S99, RVB-651	20	30	1	dirty	≥ 4	**> 4**

Note:
$TCID_{50}$- 50% infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE in 50% of cell culture units

Prepared by: Bc. Iva Čížová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D330/2016
Rep No: 34
Sample name: **Viruton Pulver**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Batch No: 20160819_55

Sampling date: 22.11.2016
Sample delivered: 28.11.2016
Testing date: 24.2. – 31.3.2017
Delivered amount: 4 x 20 g

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A1:2015)
Period of analysis:	10.3. – 20.3.2016
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Product diluent:	hard water
Appearance of the product:	white powder with blue particles
Test concentration:	1% and 2%
Contact time:	10 and 30 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K47740803613, expiry date: 31.3.2018
Test virus:	*Poliovirus* type 1, LSc-2ab (5th passage)
Cell lines:	HeLa cells
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 72 hours and

additional period of 72 hours. After incubation, the titre infectivity is calculated according to Spearman-Kärber method.

Preparation of the test

1. Determination of the number of the microorganisms CFU/ml in the product
2. Preparation of the cell culture
3. Preparation of the test virus suspension
4. Test of the viral infectivity
5. Virus titration with the interfering substance
6. Cytotoxicity of the product
7. Reference virus inactivation test
8. Test procedure for the virucidal activity of the product

Note:
Virucidal activity – the capability of a product to produce a reduction in the number of infectious virus particles under defined conditions by at least 4 (lg) orders.

The standard:
EN 14476:2013 +A1:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (Phase 2/Step 1) August 2013 + September 2015

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D330/2016
Rep No: 34
Sample name: **Viruton Pulver**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Batch No: 20160819_55

Sampling date: 22.11.2016
Sample delivered: 28.11.2016
Testing date: 24.2. – 31.3.2017
Delivered amount: 4 x 20 g

5. Testing the efficacy of chemical disinfectant **Viruton Pulver** on *Poliovirus* type 1, LSc-2ab

Tab No. 5.1 Table of results of product **Viruton Pulver** on *Poliovirus* type 1, LSc-2ab

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 10 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
Viruton Pulver	2%	clean	-	4.00	-	-
Viruton Pulver	2%	dirty	3.50	4.67	-	-
Viruton Pulver	1%	clean	-	-	4.00	-
Viruton Pulver	1%	dirty	-	-	3.67	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	7.00	5.83
			Virus titration, time = 0			
Virus control	-	PBS	9.33	-	9.33	9.17
Virus control	-	clean	9.50	9.33	9.33	-
Virus control	-	dirty	9.50	9.33	9.33	-

Tab No. 5.2 Testing the efficacy of chemical disinfectant **Viruton Pulver** on *Poliovirus* type 1, LSc-2ab

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
2%	9.50	clean	10 min	4.00	**5.50**
2%	9.50	dirty	10 min	4.67	**4.83**
1%	9.50	clean	30 min	4.00	**5.50**
1%	9.50	dirty	30 min	3.67	**5.83**

6. Evaluation of virucidal activity of the product **Viruton Pulver**

Tab No. 6.1 The efficacy of chemical disinfectant **Viruton Pulver** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013+A1:2015)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013+ A1:2015	$\Delta\log_{10}$ $TCID_{50}$
Poliovirus type 1, LSc-2ab	20	10	2	clean	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	10	2	dirty	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	30	1	clean	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	30	1	dirty	≥ 4	**> 4**

Note:
$TCID_{50}$- 50% infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE in 50% of cell culture units

Prepared by: Bc. Iva Čížová, Lab Technician

Description: *Testing the efficacy of chemical disinfectants and antiseptics*

Sample ID: D330/2016
Rep No: 34
Sample name: **Viruton Pulver**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Batch No: 20160819_55

Sampling date: 22.11.2016
Sample delivered: 28.11.2016
Testing date: 24.2. – 31.3.2017
Delivered amount: 4 x 20 g

Interpretation:
Results of tests are in Tabs.
According to EN 14476:2013+A1:2015 the tested product **Viruton Pulver,** batch No. 20160819_55, in the concentration 1%, diluted in hard water, and in the contact time 30 min under clean and dirty conditions and in the concentration 2%, diluted in hard water, and in the contact time 10 min under clean and dirty conditions at temperature 20 °C ± 1 °C **proved** by the method of virus titration on monolayers of cells on microtitre plates to reduce the number of infectious *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 particles under defined conditions by 4 (lg) orders.

According to EN 14476:2013+A1:2015 the tested product **Viruton Pulver,** batch No. 20160819_55, in the concentration 1%, diluted in hard water, and in the contact time 30 min under clean and dirty conditions and in the concentration 2%, diluted in hard water, and in the contact time 10 min under clean and dirty conditions at temperature 20 °C ± 1 °C **proved** by the method of virus titration on monolayers of cells on microtitre plates to reduce the number of infectious *Murine norovirus (MNV)* strain S99, RVB-651 particles under defined conditions by at least 4 (lg) orders.

According to EN 14476:2013+A1:2015 the tested product **Viruton Pulver,** batch No. 20160819_55, in the concentration 1%, diluted in hard water, and in the contact time 30 min under clean and dirty conditions and in the concentration 2%, diluted in hard water, and in the contact time 10 min under clean and dirty conditions at temperature 20 °C ± 1 °C **proved** by the method of virus titration on monolayers of cells on microtitre plates to reduce the number of infectious *Poliovirus* type 1, LSc-2ab, particles under defined conditions by at least 4 (lg) orders.

Conclusion:
The product **Viruton Pulver** is capable of reducing the number of infectious *Adenovirus*, *Poliovirus* and *Murine norovirus* particles under defined conditions to the declared values, and consequently, may be called virucidal.

4.4.2017, Hodonín

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Ing. Barbora Stoklásková, Leader of Study