Chemila, spol. s r.o., Za Dráhou 4386/3, Hodonín 69501, Phone +420518340919, chemila@chemila.cz
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. D202/2015

DETERMINATION OF VIRUCIDAL (EN 14476+A1) ACTIVITY OF THE PRODUCT **MEDI SPRAY** ON VACCINIA VIRUS AND HUMAN ROTAVIRUS

Sample ID: D202/2015
Sample name: **Medi Spray**
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:
26.11.2015

Delivery date:
8.1.2016

Hodonín, 8.1.2016

Ing. Jana Šlitrová, Head of Laboratory

Digitally signed by Palii Nina
Date: 2023.01.06 10:29:17 EET
Reason: MoldSign Signature
Location: Moldova

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

Sample ID: D202/2015
Rep No: 202
Sample name: **Medi Spray**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, Motycz
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 24.11.2015
Sample delivered: 26.11.2015
Testing date: 8.12. – 17.12.2015
Delivered amount: 0.5 l
Batch No: 151030-50

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

Identification of the sample:

Name of the product:	**Medi Spray**
Batch number:	151030-50
Date of manufacture:	30.10.2015
Expiry date:	30.10.2016
Manufacturer:	Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland
Incoming date:	26.11.2015
Storage conditions:	stated by the manufacturer
Active compounds and concentrations:	ethanol 55-65% propan-2-ol 5-15%

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476)
Period of analysis:	8.12. – 16.12.2015
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtiter plates
Product diluent:	hard water
Appearance of the products:	colourless liquid
Test concentration:	100% (concentrated)*
Contact time:	0.5 and 1 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: K46046503, expiry date: 2016/09/30
Test virus:	*Human rotavirus* strain WA ATCC-VR-2018 (3rd passage)
Cell lines:	African green monkey kidney (CV-1) CCL-70 cell line
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 h, and additional period of 96 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 product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and the interfering substance.

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) September 2015

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

Sample ID: D202/2015
Rep No: 202
Sample name: **Medi Spray**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, Motycz
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 24.11.2015
Sample delivered: 26.11.2015
Testing date: 8.12. – 17.12.2015
Delivered amount: 0.5 l
Batch No: 151030-50

The Number of CFU in the tested product **Medi Spray**: 0 CFU/ml

1. Testing the efficacy of chemical disinfectant **Medi Spray** on *Human rotavirus* strain WA ATCC-VR-2018

Tab No. 1.1 Table of results of product **Medi Spray** on *Human rotavirus* strain WA ATCC-VR-2018

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$TCID$_{50}$ after 0.5 min	- $\log_{10}$TCID$_{50}$ after 1 min	- $\log_{10}$TCID$_{50}$ after 30 min	- $\log_{10}$TCID$_{50}$ after 60 min
Medi Spray	100%*	clean	-	2.50	-	-	-
Medi Spray	100%*	dirty	2.50	-	2.50	-	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	-	6.83	5.83
			Virus titration, time = 0				
Virus control	-	PBS	9.50	-	-	9.33	9.33
Virus control	-	clean	9.50	9.50	-	-	-
Virus control	-	clean	9.50	-	9.50	-	-

Tab No. 1.2 Testing the efficacy of chemical disinfectant **Medi Spray** on *Human rotavirus* strain WA ATCC-VR-2018

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$_{50}$
100%*	9.50	clean	0.5 min	2.50	**7.00**
100%*	9.50	dirty	1 min	2.50	**7.00**

2. Evaluation of virucidal activity of the product **Medi Spray**

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

Virucidal activity of the product						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ TCID$_{50}$ EN 14476+A1	$\Delta\log_{10}$ TCID$_{50}$
Human rotavirus strain WA ATCC-VR-2018	20	0.5	100%*	clean	≥ 4	**> 4**
Human rotavirus strain WA ATCC-VR-2018	20	1	100%*	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
* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and the interfering substance.

Prepared by: Bc. Iva Čížová, Lab Technician

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

Sample ID: D202/2015
Rep No: 202
Sample name: **Medi Spray**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, Motycz
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 24.11.2015
Sample delivered: 26.11.2015
Testing date: 8.12. – 17.12.2015
Delivered amount: 0.5 l
Batch No: 151030-50

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476)
Period of analysis:	10.12. – 17.12.2015
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtiter plates
Appearance of the products:	colourless liquid
Test concentration:	100% (concentrated)*
Contact time:	0.5 min and 1 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: K46046503, expiry date: 2016/09/30
Test virus:	*Vacciniavirus* strain Ankara (MVA)ATCC VR-1508 (2nd passage)**
Cell lines:	BHK-21 cells (ATCC CCL-10)
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 test for virucidal activity against enveloped virus Vaccinia virus strain Ankara will cover all enveloped viruses only (Annex A, standard EN 14476:2013+A1:2015)
* The product can only be tested at a concentration of 80% or less, as some dilution is always produced by adding the inoculum and interfering substance.
** The test was performed by using MicroSpin™ S 400 HR because the virus suspension was $10^{6.5}$ $TCID_{50}$/ml

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) September 2015

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

Sample ID: D202/2015
Rep No: 202
Sample name: **Medi Spray**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, Motycz
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 24.11.2015
Sample delivered: 26.11.2015
Testing date: 8.12. – 17.12.2015
Delivered amount: 0.5 l
Batch No: 151030-50

3. Testing the efficacy of chemical disinfectant **Medi Spray** on *Vacciniavirus* strain Ankara (MVA) ATCC VR-1508 **

Tab No. 3.1 Table of results of product **Medi Spray** on *Vacciniavirus* strain Ankara (MVA) ATCC VR-1508 **

Product	Concentration	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 30 sec	- $\log_{10}$ $TCID_{50}$ after 60 sec	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
Medi Spray	100%*	clean	≤ 1.50	≤ 1.50	-	-	-	-
Medi Spray	100%*	dirty	≤ 1.50	-	≤ 1.50	-	-	-
Formaldehyde	0.7 % (w/v)	PBS	≤ 1.50	-	-	4.00	3.17	2.33
			Virus titration, time = 0					
Virus control	-	PBS	6.50	-	-	6.50	6.33	6.17
Virus control	-	clean	6.50	6.50	-	-	-	-
Virus control	-	clean	6.50	-	6.50	-	-	-

Tab No. 3.2 Testing the efficacy of chemical disinfectant **Medi Spray** on *Vacciniavirus* strain Ankara (MVA) ATCC VR-1508 **

Test concentration	Titre of the virus suspension - $\log_{10}$ $TCID_{50}$	Interfering substances	Contact time	- $\log_{10}$ $TCID_{50}$ after test procedure	$\Delta\log_{10}$ $TCID_{50}$
100%*	6.50	clean	0.5 min	≤ 1.50	**> 5.00**
100%*	6.50	dirty	1 min	≤ 1.50	**> 5.00**

4. Evaluation of virucidal activity of the product **Medi Spray**

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

Virucidal activity of the product (EN 14476)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476+A1	$\Delta\log_{10}$ $TCID_{50}$
Vacciniavirus strain Ankara (MVA) ATCC VR-1508 **	20	0.5	100*	clean	≥ 4	**> 4**
Vacciniavirus strain Ankara (MVA) ATCC VR-1508 **	20	1	100*	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
* The product can only be tested at a concentration of 80% or less, as some dilution is always produced by adding the inoculum and interfering substance.
** The test was performed by using MicroSpin™ S 400 HR because the virus suspension was $10^{6.5}$ $TCID_{50}$/ml

Prepared by: Bc. Iva Čížová, Lab Technician

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

Sample ID: D202/2015
Rep No: 202
Sample name: **Medi Spray**
Sampled: by client
Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, Motycz
Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz

Sampling date: 24.11.2015
Sample delivered: 26.11.2015
Testing date: 8.12. – 17.12.2015
Delivered amount: 0.5 l
Batch No: 151030-50
Page: 6

Interpretation:
Results of tests are in Tabs.
The tested concentrated* product **Medi Spray**, batch No: 151030-50, in the contact time 0.5 min under clean conditions and in the contact time 1 min under 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 *Human rotavirus* strain WA ATCC-VR-2018 particles under defined conditions by 4 (lg) orders (EN 14476:2013 +A1:2015).

The tested concentrated* product **Medi Spray**, batch No: 151030-50, in the contact time 0.5 min under clean conditions and in the contact time 1 min under 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 *Vacciniavirus* strain Ankara (MVA) ATCC VR-1508 ** particles under defined conditions by 4 (lg) orders (EN 14476:2013 +A1:2015).

* The product can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and the interfering substance.
** The test was performed by using MicroSpin™ S 400 HR because the virus suspension was $10^{6.5}$ $TCID_{50}$/ml
The test for virucidal activity against enveloped virus Vaccinia virus strain Ankara will cover all enveloped viruses only (Annex A, standard EN 14476:2013+A1:2015)

Conclusion:
The product **Medi Spray** is capable of reducing the number of infectious *Human rotavirus* and *Vacciniavirus* strain Ankara (MVA) ATCC VR-1508 particles under defined conditions to the declared values and, consequently, may be called virucidal on *Human rotavirus* and *Vacciniavirus*.

8.1.2016, Hodonín

Ing. Eva Kremlová, Leader of Study CZ

TESTING LABORATORY CERTIFIED BY CZECH ACCREDITATION INSTITUTE
-2-
Chemila, spol. s.r.o.
Za Dráhou 4386/3
695 01 Hodonín
CZ
č. 1273

546