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

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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

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From pages: 6

Incoming date:
26.11.2015

Delivery date:
8.1.2016

Hodonín, 8.1.2016



Ing. Jana Šlitrová, Head of Laboratory

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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

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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 % CO₂, 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

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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 cytotoxicity	- 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	Δlog 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

Strain	Test temperature [°C]	Virucidal activity of the product				
		Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	Δlog ₁₀ TCID ₅₀ EN 14476+A1	Δlog ₁₀ TCID ₅₀
<i>Human rotavirus</i> strain WA ATCC-VR-2018	20	0.5	100%*	clean	≥ 4	> 4
<i>Human rotavirus</i> strain WA ATCC-VR-2018	20	1	100%*	dirty	≥ 4	> 4

Note:

TCID₅₀- 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

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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 % CO₂, 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} TCID₅₀/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

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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 cytotoxicity	- 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	Δlog 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	Δlog ₁₀ TCID ₅₀ EN 14476+A1	Δlog ₁₀ TCID ₅₀
<i>Vacciniavirus</i> strain Ankara (MVA) ATCC VR-1508 **	20	0.5	100*	clean	≥ 4	> 4
<i>Vacciniavirus</i> strain Ankara (MVA) ATCC VR-1508 **	20	1	100*	dirty	≥ 4	> 4

Note:

TCID₅₀- 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.

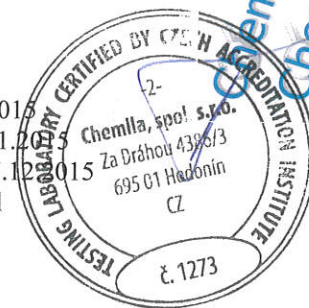
** The test was performed by using MicroSpin™ S 400 HR because the virus suspension was 10^{6.5} TCID₅₀/ml

Prepared by: Bc. Iva Čížová, Lab Technician

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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 $20\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ **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 $20\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ **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 $10^{6.5}$ TCID₅₀/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

Eva Kremlová

Ing. Eva Kremlová, Leader of Study CZ



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