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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. D103/2016

DETERMINATION OF VIRUCIDAL (EN 14476+A1) ACTIVITY OF THE  
 PRODUCT **MEDI SPRAY** ON MURINE NOROVIRUS

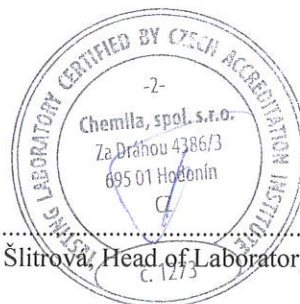
Sample ID: D103/2016  
 Sample name: **Medi Spray**  
 Client: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland  
 Producer: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland  
 Sampling point: Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland

Page: 1  
 From pages: 4

Incoming date:  
 12.5.2016

Delivery date:  
 7.6.2016

Hodonín, 7.6.2016



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 Ing. Jana Šlitrová, Head of Laboratory

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

Sample ID: D103/2016

Rep No: 79

Sample name: **Medi Spray**

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

Sampling date: 10.5.2016

Sample delivered: 12.5.2016

Testing date: 13.5. – 20.5.2016

Delivered amount: 1 l

Batch No: 16021110

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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:

16021110

Date of manufacture:

20160212

Expiry date:

20170212

Manufacturer:

Medi-Sept Sp. z o.o., Konopnica 159c, 210 30 Motycz, Poland

Incoming date:

12.5.2016

Storage conditions:

stated by the manufacturer

Active compounds and concentrations in 100 g:

63,7 g ethanol CAS 64-17-5

6,3 g propan-2-ol CAS 67-63-0

Experiment conditions:

**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method**

SOP-M-19-00 (EN 14476+A1)

Period of analysis:

13.5. – 20.5.2016

Test temperature:

20 °C ± 1 °C

Method of titration:

virus titration on monolayers of cells on microtitre plates

Appearance of the products:

colourless liquid

Test concentration:

100% (concentrated)\*

Contact time:

30 s, 60 s

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:

*Murine norovirus (MNV)* strain S99, RVB-651 (3<sup>rd</sup> passage)

Cell lines:

RAW 264.7 *Murine macrophage* cell line

Incubation:

36 °C ± 1 °C, 5 % CO<sub>2</sub>, 96 h, and additional period of 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 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 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.

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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

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

1. Testing the efficacy of chemical disinfectant **Medi Spray** on *Murine norovirus* strain S99, RVB-651

Tab No. 1.1 Table of results of product **Medi Spray** on *Murine norovirus* strain S99, RVB-651

Product	Concentration	Interfering substances	Level of cytotoxicity	- log <sub>10</sub> TCID <sub>50</sub> after 30 s	- log <sub>10</sub> TCID <sub>50</sub> after 60 s	- log <sub>10</sub> TCID <sub>50</sub> after 30 min	- log <sub>10</sub> TCID <sub>50</sub> after 60 min
<b>Medi Spray</b>	100%*	clean	≤ 1.50	3.17	3.00	-	-
<b>Medi Spray</b>	100%*	dirty	≤ 1.50	3.17	3.00	-	-
<b>Formaldehyde</b>	0.7 % (w/v)	PBS	3.50	-	-	7.33	6.50
			Virus titration, time = 0				
Virus control	-	PBS	9.50	-	-	9.33	9.17
Virus control	-	clean	9.50	9.50	9.33	-	-
Virus control	-	dirty	9.33	9.33	9.33	-	-

Tab No. 1.2 Testing the efficacy of chemical disinfectant **Medi Spray** on *Murine norovirus* strain S99, RVB-651

Test concentration	Titre of the virus suspension - log <sub>10</sub> TCID <sub>50</sub>	Interfering substances	Contact time	- log <sub>10</sub> TCID <sub>50</sub> after test procedure	Δlog <sub>10</sub> TCID <sub>50</sub>
100%*	9.50	clean	30 s	3.17	<b>6.33</b>
100%*	9.33	dirty	30 s	3.17	<b>6.16</b>
100%*	9.50	clean	60 s	3.00	<b>6.50</b>
100%*	9.33	dirty	60 s	3.00	<b>6.33</b>

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 (EN 14476)						
Strain	Test temperature [°C]	Contact time [s]	Product test concentrations [%]	Interfering substances - conditions	Δlog <sub>10</sub> TCID <sub>50</sub> EN 14476+A1	Δlog <sub>10</sub> TCID <sub>50</sub>
<i>Murine norovirus</i> strain S99, RVB-651	20	30	100*	clean	≥ 4	> 4
<i>Murine norovirus</i> strain S99, RVB-651	20	30	100*	dirty	≥ 4	> 4
<i>Murine norovirus</i> strain S99, RVB-651	20	60	100*	clean	≥ 4	> 4
<i>Murine norovirus</i> strain S99, RVB-651	20	60	100*	dirty	≥ 4	> 4

Note:

TCID<sub>50</sub>- 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.

Prepared by: Bc. Iva Čížová, Lab Technician

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Interpretation:

Results of tests are in Tabs.


According to EN 14476+A1 tested concentrated\* product **Medi Spray**, batch No. 16021110, in contact times 30 and 60 s under clean and 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 *Murine norovirus* strain S99, RVB-651, particles under defined conditions by at least 4 (lg) orders.

\* Product can only be tested at a concentration of 80% or less, as some dilution is always produced by adding the test organisms and interfering substance.

Conclusion:

The product **Medi Spray** is capable of reducing the number of infectious *Murine norovirus* particles under defined conditions to the declared values, and consequently, may be called virucidal on *Murine norovirus*.

7.6.2016, Hodonín

  
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Ing. Eva Kremlová, Leader of Study

