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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. D121/2017

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

Sample ID: D121/2017
Sample name: **Oxisept**
Client: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, Bulgaria
Manufacturer: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, Bulgaria
Sampling point: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, Bulgaria

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From pages: 8

Incoming date:
28.6.2017

Delivery date:
8.11.2017

Hodonín, 8.11.2017

Ing. Jana Šimová, Head of Laboratory
č. 1273

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Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D121/2017

Rep No: 156

Sample name: **Oxisept**

Sampled: by client

Sampling point: ZHIVAS LTD, 14, Asen Yordanov Blvd., 1592 Sofia Client:
ZHIVAS LTD, 14, Asen Yordanov Blvd., 1592 Sofia, Bulgaria

Sampling date: 15.5.2017

Sample delivered: 28.6.2017

Testing date: 19.9. - 2.10.2017

Delivered amount: 1 kg

Batch No: 087/03.2017

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Subject of testing:

Determination of virucidal activity of the product.

Identification of the sample:

Name of the product:

Oxisept

Batch number:

087/03.2017

Date of manufacture:

03.2017

Expiry date:

03.2019

Manufacturer:

ZHIVAS LTD, 14, Asen Yordanov Blvd., 1592 Sofia, Bulgaria

Incoming date:

28.6.2017

Storage conditions: r

room temperature

Active ingredients:

Peracetic acid 1000 ppm in 2-% solution, obtained "In situ" from sodium per carbonate and TAED

Peracetic acid (CAS 79-21-0), obtained "In situ" from Sodium per carbonate (CAS15630-89-4) and TAED (CAS10543-57-4)

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:

19.9. - 29.9.2017

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-green powder

Test concentration:

2.0% **

Contact time:

10 min

Interfering substances:

0.3 g/l BSA (clean 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 (5th passage)

Cell lines:

HeLa cells

Incubation:

36 °C ± 1 °C, 5 % CO₂, 120 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 test was performed by using MicroSpin™ S 400 HR

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: D121/2017

Rep No: 156

Sample name: **Oxisept**

Sampled: by client

Sampling point: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia

Client: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, Bulgaria

Sampling date: 15.5.2017

Sample delivered: 28.6.2017

Testing date: 19.9. – 2.10.2017

Delivered amount: 1 kg

Batch No: 087/03.2017

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

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

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

Product	Concentration **	Interfering substances	Level of cytotoxicity	$-\log_{10}$ TCID ₅₀ after 10 min	$-\log_{10}$ TCID ₅₀ after 30 min	$-\log_{10}$ TCID ₅₀ after 60 min
Oxisept	2.0%	clean	3.50	4.67	-	-
Formaldehyde	0.7 % (w/v)	PBS	≤ 1.50	-	6.33	5.00
			Virus titration, time = 0			
Virus control	-	PBS	9.50	-	9.33	9.33
Virus control	-	clean	9.50	9.50	-	-

Tab No. 1.2 Testing the efficacy of chemical disinfectant **Oxisept** 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
2.0%	9.50	clean	10 min	4.67	4.83

2. Evaluation of virucidal activity of the product **Oxisept**

Tab No. 2.1 The efficacy of chemical disinfectant **Oxisept** 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
<i>Adenovirus</i> type 5, strain Adenoid 75, ATCC VR-5	20	10	2.0	clean	≥ 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 test was performed by using MicroSpin™ S 400 HR

Prepared by: Bc. Iva Čížová, Lab Technician

Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D121/2017

Rep No: 156

Sample name: **Oxisept**

Sampled: by client

Sampling point: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia

Client: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, Bulgaria

Sampling date: 15.5.2017

Sample delivered: 28.6.2017

Testing date: 19.9. – 2.10.2017

Delivered amount: 1 kg

Batch No: 087/03.2017

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Experiment conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method SOP-M-19-00 (EN 14476:2013 +A1:2015)

Period of analysis:

22.9. – 2.10.2017

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-green powder

Test concentration:

2.0% **

Contact time:

10 min

Interfering substances:

0.3 g/l BSA (clean 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 % CO₂, 120 h, and additional period of 120 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 was performed by using MicroSpin™ S 400 HR

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: D121/2017

Rep No: 156

Sample name: **Oxisept**

Sampled: by client

Sampling point: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia

Client: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, Bulgaria

Sampling date: 15.5.2017

Sample delivered: 28.6.2017

Testing date: 19.9. – 2.10.2017

Delivered amount: 1 kg

Batch No: 087/03.2017

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

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

Product	Concentration **	Interfering substances	Level of cytotoxicity	- log ₁₀ TCID ₅₀ after 10 min	- log ₁₀ TCID ₅₀ after 30 min	- log ₁₀ TCID ₅₀ after 60 min
Oxisept	2.0%	clean	3.50	5.00	-	-
Formaldehyde	0.7 % (w/v)	PBS	≤1.50	-	7.67	6.17
			Virus titration, time = 0			
Virus control	-	PBS	10.00	-	9.83	9.83
Virus control	-	clean	10.00	10.00	-	-

Tab No. 3.2 Testing the efficacy of chemical disinfectant **Oxisept** 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	Δlog ₁₀ TCID ₅₀
2.0%	10.00	clean	10 min	5.00	5.00

4. Evaluation of virucidal activity of the product **Oxisept**

Tab No. 4.1 The efficacy of chemical disinfectant **Oxisept** 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	Δlog ₁₀ TCID ₅₀ EN 14476:2013+A1:2015	Δlog ₁₀ TCID ₅₀
<i>Murine norovirus (MNV)</i> strain S99, RVB-651	20	10	2.0	clean	≥ 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 test was performed by using MicroSpin™ S 400 HR

Prepared by: Bc. Iva Čížová, Lab Technician

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

Sample ID: D121/2017

Rep No: 156

Sample name: **Oxisept**

Sampled: by client

Sampling point: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia

Client: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, Bulgaria

Sampling date: 15.5.2017

Sample delivered: 28.6.2017

Testing date: 19.9. – 2.10.2017

Delivered amount: 1 kg

Batch No: 087/03.2017

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Experiment conditions:

Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method SOP-M-19-00

(EN 14476:2013 +A1:2015)

Period of analysis:

20.9. – 26.9.2017

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-green powder

Test concentration:

2.0% **

Contact time:

10 min

Interfering substances:

0.3 g/l BSA (clean 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 % CO₂, 96 h, 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 test was performed by using MicroSpin™ S 400 HR

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: D121/2017

Rep No: 156

Sample name: **Oxisept**

Sampled: by client

Sampling point: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia

Client: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, Bulgaria

Sampling date: 15.5.2017

Sample delivered: 28.6.2017

Testing date: 19.9. – 2.10.2017

Delivered amount: 1 kg

Batch No: 087/03.2017

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

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

Product	Concentration **	Interfering substances	Level of cytotoxicity	- log ₁₀ TCID ₅₀ after 10 min	- log ₁₀ TCID ₅₀ after 30 min	- log ₁₀ TCID ₅₀ after 60 min
Oxisept	2.0%	clean	3.50	4.67	-	-
Formaldehyde	0.7 % (w/v)	PBS	≤1.50	-	7.00	5.67
			Virus titration, time = 0			
Virus control	-	PBS	9.50	-	9.50	9.33
Virus control	-	clean	9.50	9.50	-	-

Tab No. 5.2 Testing the efficacy of chemical disinfectant **Oxisept** 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	Δlog ₁₀ TCID ₅₀
2.0%	9.50	clean	10 min	4.67	4.83

6. Evaluation of virucidal activity of the product **Oxisept**

Tab No. 6.1 The efficacy of chemical disinfectant **Oxisept** 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	Δlog ₁₀ TCID ₅₀ EN 14476:2013+A1:2015	Δlog ₁₀ TCID ₅₀
<i>Poliovirus</i> type 1, LSc-2ab	20	10	2.0	clean	≥ 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 test was performed by using MicroSpin™ S 400 HR

Prepared by: Bc. Iva Čížová, Lab Technician

Description: Testing the efficacy of chemical disinfectants and antiseptics

Sample ID: D121/2017

Rep No: 156

Sample name: **Oxisept**

Sampled: by client

Sampling point: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia

Client: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, Bulgaria

Sampling date: 15.5.2017

Sample delivered: 28.6.2017

Testing date: 19.9. – 2.10.2017

Delivered amount: 1 kg

Batch No: 087/03.2017

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Interpretation:

Results of tests are in Tabs.

According to EN 14476:2013+A1:2015 the tested product **Oxisept**, batch No. 087/03.2017, in the concentration 2.0%**, diluted in hard water, and in the contact time 10 min under clean 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 *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 particles under defined conditions by at least 4 (lg) orders.

According to EN 14476:2013+A1:2015 the tested product **Oxisept**, batch No. 087/03.2017, in the concentration 2.0%**, diluted in hard water, and in the contact time 10 min under clean 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 (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 **Oxisept**, batch No. 087/03.2017, in the concentration 2.0%**, diluted in hard water, and in the contact time 10 min under clean 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 *Poliovirus* type 1, LSc-2ab, particles under defined conditions by at least 4 (lg) orders.

** The test was performed by using MicroSpin™ S 400 HR

Conclusion:

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

8.11.2017, Hodonín

Mgr. Mirka Horáková, PhD., Leader of Study

