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Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute according to ČSN EN ISO/IEC 17025:2005.

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Copy No.: 1
Issue No.: 1

Test report No. S307/2018

DETERMINATION OF VIRUCIDAL (EN 14476:2013+A1:2015) ACTIVITY OF THE PRODUCT **GLOBACID AF**

Sample ID: S307/2018
Sample name: **GLOBACID AF**
Client: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Producer: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Sampling point: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404

Incoming date:
12.11.2018

Delivery date:
18.12.2018

Hodonín, 18.12.2018

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Ing. Jana Šlitrová, Head of Laboratory

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

Sample ID: S307/2018
Rep No: 181
Sample name: **GLOBACID AF**
Sampled: by client
Sampling point: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Client: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Batch No: 170473

Sampling date: 9.11.2018
Sample delivered: 12.11.2018
Testing date: 28.11. – 14.12.2018
Delivered amount: 400 ml

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

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Identification of the sample:

Name of the product:	**GLOBACID AF**
Batch number:	170473
Date of manufacture:	06.2017
Expiry date:	06.2020
Manufacturer:	Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Incoming date:	12.11.2018
Storage conditions:	stated by the manufacturer
Active ingredients:	CAS: 67-63-0 Isopropanol 50%-65% CAS: 64-17-5 Ethanol 10% - 20%

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:	28.11. – 7.12.2018
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:	colourless liquid
Test concentration:	100%(80%) */**
Contact time:	1 min and 3 min
Interfering substances:	0.3 g/l BSA (clean conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K50163503815, expiry date: 30.4.2020
Test virus:	*Murine norovirus (MNV)* strain S99, RVB-651 (3rd passage)
Cell lines:	RAW 264.7 *Murine macrophage* cell line
Incubation:	36 °C ± 1 °C, 5 % CO_2, 96 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 a 4 lg reduction.
* 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.
** The test was performed by using MicroSpin™ S 400 HR.

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

Sample ID: S307/2018
Rep No: 181
Sample name: **GLOBACID AF**
Sampled: by client
Sampling point: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Client: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Batch No: 170473

Sampling date: 9.11.2018
Sample delivered: 12.11.2018
Testing date: 28.11. – 14.12.2018
Delivered amount: 400 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) August 2013 + September 2015

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

Tab No. 1.1 Table of results of product **GLOBACID AF** on *Murine norovirus (MNV)* strain S99, RVB-651

Product	Concentration **	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 1 min	- $\log_{10}$ $TCID_{50}$ after 3 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
GLOBACID AF	100%*	clean	3.50	4.50	4.00	-	-
Formaldehyde	0.7 % (w/v)	PBS	4.50	-	-	7.17	6.50
			Virus titration, time = 0				
Virus control	-	PBS	9.00	-	-	9.00	8.83
Virus control	-	clean	9.00	9.00	9.00	-	-

Tab No. 1.2 Testing the efficacy of chemical disinfectant **GLOBACID AF** 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	$\Delta\log_{10}$ $TCID_{50}$
100%*	9.00	clean	1 min	4.50	**4.50**
100%*	9.00	clean	3 min	4.00	**5.00**

2. Evaluation of virucidal activity of the product **GLOBACID AF**

Tab No. 2.1 The efficacy of chemical disinfectant **GLOBACID AF** 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	1	100*	clean	≥ 4	**> 4**
Murine norovirus (MNV) strain S99, RVB-651	20	3	100*	clean	≥ 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 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: S307/2018
Rep No: 181
Sample name: **GLOBACID AF**
Sampled: by client
Sampling point: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Client: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Batch No: 170473

Sampling date: 9.11.2018
Sample delivered: 12.11.2018
Testing date: 28.11. – 14.12.2018
Delivered amount: 400 ml

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:	6.12. – 14.12.2018
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:	colourless liquid
Test concentration:	100%(80%) */**
Contact time:	1 min and 3 min
Interfering substances:	0.3 g/l BSA (clean conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K50163503815, expiry date: 30.4.2020
Test virus:	*Poliovirus* type 1, LSc-2ab (2nd passage)
Cell lines:	HeLa cells
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 a 4 lg reduction.
* 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.
** 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: S307/2018
Rep No: 181
Sample name: **GLOBACID AF**
Sampled: by client
Sampling point: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Client: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Batch No: 170473

Sampling date: 9.11.2018
Sample delivered: 12.11.2018
Testing date: 28.11. – 14.12.2018
Delivered amount: 400 ml

3. Testing the efficacy of chemical disinfectant **GLOBACID AF** on *Poliovirus* type 1, LSc-2ab

Tab No. 3.1 Table of results of product **GLOBACID AF** on *Poliovirus* type 1, LSc-2ab

Product	Concentration **	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 1 min	- $\log_{10}$ $TCID_{50}$ after 3 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
GLOBACID AF	100%*	clean	3.50	4.33	4.00	-	-
Formaldehyde	0.7 % (w/v)	PBS	4.50	-	-	6.83	5.83
			Virus titration, time = 0				
Virus control	-	PBS	9.00	-	-	9.00	9.17
Virus control	-	clean	9.00	9.00	9.00	-	-

Tab No. 3.2 Testing the efficacy of chemical disinfectant **GLOBACID AF** 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	$\Delta\log_{10}$ $TCID_{50}$
100%*	9.00	clean	1 min	4.33	**4.67**
100%*	9.00	clean	3 min	4.00	**5.00**

4. Evaluation of virucidal activity of the product **GLOBACID AF**

Tab No. 4.1 The efficacy of chemical disinfectant **GLOBACID AF** 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	1	100*	clean	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	3	100*	clean	≥ 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
* 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.
** 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: S307/2018
Rep No: 181
Sample name: **GLOBACID AF**
Sampled: by client
Sampling point: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Client: Goodpoint Chemicals Ltd, Urda tee 3, Jälgimäe, Saku vald, Harjumaa, Estonia 76404
Batch No: 170473

Sampling date: 9.11.2018
Sample delivered: 12.11.2018
Testing date: 28.11. – 14.12.2018
Delivered amount: 400 ml

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Interpretation:
Results of tests are in Tabs.
According to EN 14476:2013+A1:2015 the tested concentrated*/** product **GLOBACID AF**, batch No. 170473, in the contact times 1 min and 3 min under clean conditions at temperature 20 °C ± 1 °C **proved** by the method of virus titration on monolayers of cells on microtiter plates to reduce the number of infectious *Murine norovirus (MNV)* strain S99, RVB-651 particles under defined conditions by at least a 4 lg reduction.

According to EN 14476:2013+A1:2015 the tested concentrated*/** product **GLOBACID AF**, batch No. 170473, in the contact times 1 min and 3 min under clean 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 a 4 lg reduction.

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

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

18.12.2018, Hodonín

Ing. Eva Kremlová, Leader of Study

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Raw data – product **Globacid AF** tested against *Murine norovirus (MNV)* strain S99, RVB-651

Sample S307/2018, the test report S307/2018,

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period of analysis: 28.11. – 7.12.2018

EN14476+A1: *Murine norovirus (MNV)* strain S99, RVB-651 – 3rd passage (FLI Germany, 15.12.2010), RAW 264.7 *Murine macrophage* cell line – 3rd passage (LGC Standards Sp. z o.o., PL, 23.8. 2016)

the test conditions: 100%(80%)*/**, 1 min, 3 min, clean conditions, 20 °C

Interfering substances: 0.3 g/l BSA (clean conditions)
Reference product: Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K50163503815, expiry date: 30.4.2020

****Using Microspin**

Product	Concentration	Interfering substance	Contact time min	Dilution								
				2	3	4	5	6	7	8	9	10
Globacid AF	100%(80%)	clean	1	n.a.	444 444	222 222	000 000	000 000	000 000	000 000	000 000	000 000
Globacid AF	100%(80%)	clean	3	n.a.	444 444	022 200	000 000	000 000	000 000	000 000	000 000	000 000
Globacid AF cytotoxicity	100%(80%)	clean	n.a.	n.a.	444 444	000 000	000 000	000 000	n.d.	n.d.	n.d.	n.d.
Formaldehyde	0.7 (w/v)	PBS	30	n.a.	444 444	444 444	322 333	222 222	022 220	000 000	000 000	000 000
			60	n.a.	444 444	444 444	333 323	222 222	000 000	000 000	000 000	000 000
Formaldehyde cytotoxicity	0.7 (w/v)	PBS	n.a.	n.a.	444 444	444 444	000 000	000 000	000 000	000 000	000 000	000 000
Interference control	non-cytotoxic concentration	n.a.	n.a.	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	222 200	000 000
Neutralization	100%(80%)	clean	n.a.	n.d.	n.d.	444 444	444 444	333 333	222 222	222 222	n.d.	n.d.
Virus control	n.a.	PBS	0	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	000 222	000 000
			30	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	002 220	000 000
			60	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	200 020	000 000
Virus control	n.a.	clean	0	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	002 220	000 000
			1	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	002 220	000 000
			3	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	202 020	000 000

n.a. – not available
n.d. – not done
* 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.
** The test was performed by using MicroSpin™ S 400 HR.

Prepared by: Bc. Iva Čížová, Lab Technician
Controlled by: Ing. Eva kremlová, Leader of Study

Raw data – product **Globacid AF** tested against *Poliovirus* type 1, LSc-2ab

Sample S307/2018, the test report S307/2018,

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period of analysis: 6.12. – 14.12.2018

EN14476+A1: *Poliovirus* type 1, LSc-2ab – 2nd passage (NIBSC, GB, 28.3.2018),
HeLa cells – 3rd passage (LGC Standards Sp. z o.o., PL, 1.10. 2014)

the test conditions: 100%(80%)*/**, 1 min, 3 min, clean conditions, 20 °C

Interfering substances: 0.3 g/l BSA (clean conditions)
Reference product: Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K50163503815, expiry date: 30.4.2020

**Using Microspin

Product	Concentration	Interfering substance	Contact time min	Dilution								
				2	3	4	5	6	7	8	9	10
Globacid AF	100%(80%)	clean	1	n.a.	444 444	222 000	022 000	000 000	000 000	000 000	000 000	000 000
Globacid AF	100%(80%)	clean	3	n.a.	444 444	000 222	000 000	000 000	000 000	000 000	000 000	000 000
Globacid AF cytotoxicity	100%(80%)	clean	n.a.	n.a.	444 444	000 000	000 000	000 000	n.d.	n.d.	n.d.	n.d.
Formaldehyde	0.7 (w/v)	PBS	30	n.a.	444 444	444 444	322 322	222 222	020 020	000 000	000 000	000 000
			60	n.a.	444 444	444 444	222 232	002 200	000 000	000 000	000 000	000 000
Formaldehyde cytotoxicity	0.7 (w/v)	PBS	n.a.	n.a.	444 444	444 444	000 000	000 000	000 000	000 000	000 000	000 000
Interference control	non-cytotoxic concentration	n.a.	n.a.	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	000 222	000 000
Neutralization	100%(80%)	clean	n.a.	n.d.	n.d.	444 444	444 444	333 333	222 222	222 222	n.d.	n.d.
Virus control	n.a.	PBS	0	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	022 200	000 000
			30	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	002 220	000 000
			60	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	220 022	000 000
Virus control	n.a.	clean	0	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	222 000	000 000
			1	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	202 020	000 000
			3	n.a.	444 444	444 444	444 444	333 333	222 222	222 222	022 200	000 000

n.a. – not available
n.d. – not done
* 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.
** The test was performed by using MicroSpin™ S 400 HR.

Prepared by: Bc. Iva Čížová, Lab Technician
Controlled by: Ing. Eva kremlová, Leader of Study