Department of Biocidal Products Implementation
Ekolabos sp. z o.o. (Ltd)
Duńska Street 9
54-427 Wrocław

Update No. 1 of the report: **RB/4019/11/20**

Update 1 issue date: **12th of July 2022**

Antibacterial efficacy assessment report

for the product

Velox Oxy ETA

according to PN-EN 13727+A2:2015-12 standard

made for the company

MEDISEPT Sp. z o.o.

Konopnica 159 c,

21-030 Motycz, Poland

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Comments: Update No. 1 of the report prepared at the request of the client - adding information on the concentration of active substances.

The presented measurement results refer to the tested objects solely.

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1. INTRODUCTION

The properties of biocidal preparations, before they are authorised for use, are assessed based on tests carried out in accordance with European standards or other methods accepted by designated national authorities.

The standardisation of testing methods in recent years, through the development of successive European standards on the efficacy of disinfectants and antiseptics, allows a uniform, objective assessment of the antimicrobial effect of these agents and guarantees that the products offered in the market have adequate efficacy.

2. PURPOSE OF THE STUDY

The study aims to assess the antibacterial efficacy of the product in relation to *Escherichia coli* K12 NCTC 10538, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442 and *Enterococcus hirae* ATCC 10541 strains.

3. FORMAL BASIS

The assessment of antibacterial efficacy was carried out on the basis of the agreement/order dated 6th of November 2020 (Agreement No.: AFC/020784/11/20/WRO) concluded between the Contracting

Party and the Contractor.

Contracting Party:

MEDISEPT Sp. z o.o.

Konopnica 159 c,

21-030 Motycz, Poland

Contractor:

EKOLABOS sp. z o. o.

Environmental Research Laboratory

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4. LEGAL BASIS

The legal basis for the conducted tests is:

The Act of 9 October 2015 on biocidal products

PN-EN 13727+A2:2015-12 Chemical disinfectants and antiseptics – quantitative suspension method for the evaluation of antibacterial effect in the medical area. Test method and requirements (phase 2, stage 1). According to the standard, the disinfectant has an antibacterial effect on the strain used if the logarithm for bacterial cell reduction obtained in the test is ≥5.

5. SAMPLE IDENTIFICATION[1]

The tested sample was the biocidal product in the form of a ready-to-use biocidal product. The preparation was accepted for testing on 9th of November 2020. Sample code assigned by the laboratory: 005/09/11/20.

Product name: Velox Oxy ETA

Batch No.: no data

Product reference number: no data

Manufacturer:

MEDISEPT Sp. z o.o.

Konopnica 159 c,

21-030 Motycz, Poland

Date of manufacture: no data

Expiry date: no data

Product appearance: clear, colorless liquid

Recommended product solvent: N/A

Storage conditions: no data

Active substances present in the product provided by the Contracting Party and their concentrations:

- Ethanol (CAS: 64-17-5) - 72.8g
- Propan-2-ol (CAS: 67-63-0) - 7.2g
- Hydrogen peroxide (CAS: 7722-84-1) -3g

[1] Declaration by the Principal

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6. SCOPE OF TASKS PERFORMED

Phase 2, stage 1 assessment consists in using the dilution and neutralisation method in which the test organism is exposed to the preparation at different concentrations, time and temperatures with the addition of aggravating substances. These methods are to confirm the efficacy of the product in laboratory conditions, similar to the intended use.

6.1 CONDITIONS OF THE TEST PERFORMED

Tests performed on: 10th of November 2020-16th of November 2020

Identification of the bacterial strains:

Escherichia coli K12 NCTC 10538,

Staphylococcus aureus ATCC 6538,

Pseudomonas aeruginosa ATCC 15442,

Enterococcus hirae ATCC 10541.

Incubation for 24h at 37 °C ± 1 °C

Number of times the test is repeated on the microbe: 1

Required test temperature: 20 °C ± 1 °C

Duration of the product contacting the bacterial suspension: 15 s ± 5 s, 30 s ± 5 s.

interfering substances: beef albumin 3g/l + 3ml/l erythrocytes

Solvent used during the test:

Hard Water according to PN-EN 13727+A2:2015-12 standard

Stability of the product during the test:

no precipitate during testing

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6.2 TESTING METHOD AND VALIDATION

Method used: neutralisation of solutions

Counting method: deep inoculation on plates

Neutraliser used, composition:

Polysorbate 80 – 30 g/l

Sodium thiosulphate – 10 g/l

Lecithin – 3 g/l

The neutraliser used allowed for validation of the method.

Substrate used: Tryptone Soya Agar (TSA)

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7. TESTS RESULTS

The results of product testing are presented in tables 1-2.

Table 1. Results of validation tests

Test organism	Test bacterial suspension	Validation bacterial suspension	Validation test	Control of neutraliser toxicity	Test using water
	N	**Nv_0**	**A**	**B**	**C**
Staphylococcus aureus ATCC 6538	10^{-6}: >330 10^{-7}: 46 **N: 8,66**	**Nv_0: 104**	**A: 95**	**N_{vB}:9,9x10^4 B: 95**	**C: 96**
Pseudomonas aeruginosa ATCC 15442	10^{-6}: >330 10^{-7}: 56 **N: 8,56**	**Nv_0: 91**	**A: 84**	**N_{vB}:9,7x10^4 B: 87**	**C: 83**
Enterococcus hirae ATCC 10541	10^{-6}: >330 10^{-7}: 68 **N: 8,58**	**Nv_0: 96**	**A: 91**	**N_{vB}:9,8x10^4 B: 95**	**C: 90**
Escherichia coli K12 NCTC 10538	10^{-6}: >330 10^{-7}: 41 **N: 8,61**	**Nv_0: 89**	**A: 84**	**N_{vB}:8,5x10^4 B: 81**	**C: 85**

N – log from the number of CFU/ml put in the test suspension
Nv_0 – 1/10th of number of CFU/ml in validation suspension
Nv_b – 1/10th of number of CFU/ml in validation suspension for neutraliser toxicity test
A – number of CFU/ml in the mixture to be validated
B – number of CFU/ml in the mixture for neutraliser toxicity test
C - number of CFU/ml in the mixture to be controlled using water and the highest concentration of the active substance

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Table 2. Test results

Test organism	N_0	Results for individual concentrations of the product in volumetric % (test conditions: contact time: 15 sec and 30 sec, temperature: 20°C ± 1°C)		
		80%	**1%**	**0,1%**
Staphylococcus aureus ATCC 6538	**7,66**	<14, <14	>330, >330	>330, >330
		Na: <140 Log Na: >2,15	**Na: >3300 Log Na: >3,52**	**Na: >3300 Log Na: >3,52**
R (N_0 – Log Na)		**R: >5,52**	**R: <4,14**	**R: <4,14**
Pseudomonas aeruginosa ATCC 15442	**7,56**	<14, <14	>330, >330	>330, >330
		Na: <140 Log Na: <2,15	**Na: >3300 Log Na: >3,52**	**Na: >3300 Na: >3,52**
R (N_0 – Log Na)		**R:>5,41**	**R:<4,04**	**R:<4,04**
Enterococcus hirae ATCC 10541	**7,68**	<14, <14	>330, >330	>330, >330
		Na: <140 Log Na: <2,15	**Na: >3300 Log Na: >3,52**	**Na: >3300 Na: >3,52**
R (N_0 – Log Na)		**R:>5,54**	**R:<4,16**	**R:<4,16**
Escherichia coli K12 NCTC 10538	**7,61**	<14, <14	>330, >330	>330, >330
		Na: <140 Log Na: <2,15	**Na: >3300 Log Na: >3,52**	**Na: >3300 Na: >3,52**
R (N_0 – Log Na)		**R:>5,47**	**R: <4,09**	**R: <4,09**

N_0 – log (N/10)
Na – number of CFU/ml in the test mixture after treatment
R – logarithm bacterial cell reduction obtained during the test

Specific comments:

Verification of methodology – requirements and limits:

- N is between $1.5x10^8$ CFU/ml and $5x10^8$ CFU/ml ($8,17 \leq \log N \leq 8,70$),
- N_0 is between $1.5x10^7$ CFU/ml and $5x10^7$ CFU/ml ($7,17 \leq \log N0 \leq 7,70$),
- N_{V0} is between 30 CFU/ml and 160 CFU/ml
- N_{VB} is between $3.0x10^4$ CFU/ml and $1.6x10^5$ CFU/ml
- N_V is between $3.0 x10^2$ CFU/ml and $1.6 x10^3$ CFU/ml
- The control of the weighted average from the successive dilutions is between 5.0 and 15.0
- The average number of bacteria and yeasts, on each plate used for the calculation and obtained from the active concentration test, is between 14 and 330
- A and C are equal to or greater than $0.5xN_{V0}$
- B is equal to or greater than $0.0005xN_{Vb}$
- At least one test concentration of the product must show a reduction log ≥5 and at least one test concentration of the product must show a reduction log <5.

8. CONCLUSIONS

The product, tested in accordance with PN-EN 13727+A2:2015-12 standard, after a contact time of 15 sec and 30 sec at 20°C, in the presence of an aggravating substance, shows antibacterial effect (reduction ≥5 log) in relation to:

Staphylococcus aureus	ATCC 6538	at 80% concentration
Pseudomonas aeruginosa	ATCC 15442	at 80% concentration
Enterococcus hirae	ATCC 10541	at 80% concentration
Escherichia coli K12	NCTC 10538	at 80% concentration

The results obtained during all controls and tests met all the requirements of the methodology and were within the limits set.

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The results were authorised by: Agnieszka Pawelec, M.Sc

The report was approved by: Mateusz Latosiński, Eng

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