

Report No.: **RB/4947/02/21**

Date issued: 22 March 2021

Antibacterial efficacy assessment report
for the product

Oxine Pro 500ppm

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

made for the company

EUROPEAN HYGIENE TECH AS

Skjergardsvegen 333

5353 Straume, Norway

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The presented results of the analysis refer only to the product tested.

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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 26 November 2020 (Agreement No.: AFC/022361/02/21/WRO) concluded between the Contracting Party and the Contractor.

Contracting Party:

EUROPEAN HYGIENE TECH AS
Skjergardsvegen 333
5353 Straume, Norway

Contractor:

EKOLABOS sp. z o.o.
Environmental Research Laboratory
ul. Duńska 9 54-427 Wrocław
Poland

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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 .

Guidance on the Biocidal Products Regulation - Volume II Efficacy - Assessment and Evaluation (Parts B+C), Version 3.0, April 2018.

Regulation of the European Parliament and of the Council (EU) No. 528/2012 of

22 May 2012 concerning the making available on the market and use of biocidal products.

5. SAMPLE IDENTIFICATION¹

The tested sample was the biocidal product in the form of a ready-to-use liquid. The preparation was accepted for testing on 10 February 2021. Sample code assigned by the laboratory: 085/10/02/21.

Product name: Oxine Pro 500ppm

Batch No.: 25012021

Product reference number: 25012021

Manufacturer: Biocide International - EUROPEAN HYGIENE TECH AS.

Date of manufacture: 25012021

Expiry date: 12 mnd. 25.01.2022

Product appearance: liquid, light green, light smell

Recommended product solvent: N/A

Storage conditions: cold and dark

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

- information in SDS

¹ Declaration by the Principal



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: 11 03 2021 – 18 03 2021

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: 60 sec ± 5 sec, 120 sec ± 5 sec

interfering substances beef albumin 0.3 g/l, beef albumin 3 g/l plus 3.0 ml/l erythrocytes

Solvent used during the test:

Demineralised water

Stability of product mixture with solvent:

no precipitation formed during the test

6.2 TESTING METHOD AND VALIDATION

Method used: neutralisation of solutions

Counting method: deep inoculation on plates

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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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Signed by QES



7. TESTS RESULTS

The results of product testing are presented in tables 1-4.

Table 1. Results of validation tests of clean conditions

Test organism	Test bacterial suspension	Validation bacterial suspension	Validation test	Control of neutraliser toxicity	Test using water
	N	Nv₀	A	B	C
<i>Staphylococcus aureus</i> ATCC 6538	10 ⁻⁶ : >330	Nv₀: 94	A: 91	N_{vB}: 8,6x10⁴ B: 84	C: 92
	10 ⁻⁷ : 39				
	N: 7,59				
<i>Pseudomonas aeruginosa</i> ATCC 15442	10 ⁻⁶ : >330	Nv₀: 87	A: 84	N_{vB}: 8,2x10⁴ B: 79	C: 85
	10 ⁻⁷ : 42				
	N: 7,62				
<i>Enterococcus hirae</i> ATCC 10541	10 ⁻⁶ : >330	Nv₀: 82	A: 78	N_{vB}: 9,1x10⁴ B: 90	C: 80
	10 ⁻⁷ : 39				
	N: 8,59				
<i>Escherichia coli</i> K12 NCTC 10538	10 ⁻⁶ : >330	Nv₀: 79	A: 76	N_{vB}: 9,4x10⁴ B: 91	C: 75
	10 ⁻⁷ : 40				
	N: 8,60				

N – log from the number of CFU/ml put in the test suspension

Nv₀ – 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 of clean conditions

Test organism	N ₀	Results for individual concentrations of the product in volumetric % (test conditions: contact time: 60 seconds, temperature: 20°C ± 1°C, clean conditions)		
		100%	1%	0,1%
Staphylococcus aureus ATCC 6538	7,59	<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: >5,44	R: <4,07	R: <4,07
Pseudomonas aeruginosa ATCC 15442	7,62	<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:>5,48	R:<4,10	R:<4,10
Enterococcus hirae ATCC 10541	7,59	<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:>5,44	R:<4,07	R:<4,07
Escherichia coli K12 NCTC 10538	7,60	<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:>5,46	R: <4,08	R: <4,08

N₀ – log (N/10)

Na – number of CFU/ml in the test mixture after treatment

R – logarithm bacterial cell reduction obtained during the test

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Table 3. Results of validation tests of dirty conditions

Test organism	Test bacterial suspension	Validation bacterial suspension	Validation test	Control of neutraliser toxicity	Test using water
	N	Nv ₀	A	B	C
<i>Staphylococcus aureus</i> ATCC 6538	10 ⁻⁶ : >330	Nv ₀ : 92	A: 87	N _{vB} : 8,9x10 ⁴ B: XX	C: 90
	10 ⁻⁷ : 41				
	N: 8,61				
<i>Pseudomonas aeruginosa</i> ATCC 15442	10 ⁻⁶ : >330	Nv ₀ : 87	A: 82	N _{vB} : 8,4x10 ⁴ B: 81	C: 84
	10 ⁻⁷ : 43				
	N: 8,63				
<i>Enterococcus hirae</i> ATCC 10541	10 ⁻⁶ : >330	Nv ₀ : 85	A: 84	N _{vB} : 9,2x10 ⁴ B: 89	C: 81
	10 ⁻⁷ : 38				
	N: 8,58				
<i>Escherichia coli</i> K12 NCTC 10538	10 ⁻⁶ : >330	Nv ₀ : 76	A: 75	N _{vB} : 9,1x10 ⁴ B: 88	C: 74
	10 ⁻⁷ : 39				
	N: 8,59				

N – log from the number of CFU/ml put in the test suspension

Nv₀ – 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 4. Test results of dirty conditions

Test organism	N ₀	Results for individual concentrations of the product in volumetric % (test conditions: contact time: 120 seconds, temperature: 20°C ± 1°C, dirty conditions)		
		100%	1%	0,1%
Staphylococcus aureus ATCC 6538	7,61	<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: >5,47	R: <4,09	R: <4,09
Pseudomonas aeruginosa ATCC 15442	7,63	<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:>5,49	R:<4,11	R:<4,11
Enterococcus hirae ATCC 10541	7,58	<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:>5,43	R:<4,06	R:<4,06
Escherichia coli K12 NCTC 10538	7,59	<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:>5,44	R: <4,07	R: <4,07

N₀ – log (N/10)

Na – number of CFU/ml in the test mixture after treatment

R – logarithm bacterial cell reduction obtained during the test

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Specific comments:

Verification of methodology – requirements and limits:

- N is between 1.5×10^8 CFU/ml and 5×10^8 CFU/ml ($8,17 \leq \log N \leq 8,70$),
- N_0 is between 1.5×10^7 CFU/ml and 5×10^7 CFU/ml ($7,17 \leq \log N_0 \leq 7,70$),
- N_{V0} is between 30 CFU/ml and 160 CFU/ml
- N_{VB} is between 3.0×10^4 CFU/ml and 1.6×10^5 CFU/ml
- N_V is between 3.0×10^2 CFU/ml and 1.6×10^3 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 $0.5 \times N_{V0}$
- B is equal to or greater than $0.0005 \times N_{VB}$
- At least one test concentration of the product must show a reduction $\log \geq 5$ 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 standard, during 60 and 120 seconds at 20°C, in the presence of an aggravating substance, shows antibacterial effect (reduction ≥ 5 log) in relation to:

<i>Staphylococcus aureus</i>	ATCC 6538	at 100% concentration
<i>Pseudomonas aeruginosa</i>	ATCC 15442	at 100% concentration
<i>Enterococcus hirae</i>	ATCC 10541	at 100% concentration
<i>Escherichia coli</i> K12	NCTC 10538	at 100% 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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Date issued: 22 March 2021

Report prepared by: Agnieszka Pawelec, M.Sc

The results were authorised by: Mateusz Latosiński, Eng

--- END OF REPORT ---

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