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Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute according to ČSN EN ISO/IEC 17025:2018.

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Issue No.: 1

Test report No.: S172/2023 - 1

DETERMINATION OF SPORICIDAL (EN 17126:2018)
ACTIVITY OF THE PRODUCT
OXISEPT

Sample ID: S172/2023
Sample name: **OXISEPT**
Client: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, BG
Manufacturer: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, BG
Sampling point: ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, BG

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Incoming date:
19.9.2023

Delivery date:
22.11.2023

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Sampling date: not available
Sample delivered: 19.9.2023
Testing date: 8.11. - 13.11.2023
Delivered amount: 2x 500 ml
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Determination of sporicidal activity of the product.

Name of the product:	OXISEPT
Batch number (Lot):	184/09.2023
Date of manufacture:	09/2023
Expiry date:	08/2025
Manufacturer:	ZHIVAS LTD, 14, Assen Yordanov Blvd., 1592 Sofia, BG
Incoming date:	19.9.2023
Storage conditions:	room temperature, dark area
Active ingredients:	CAS:79-21-0, Peracetic acid, 0.15 g/100 g

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

SOP:	SOP-M-19-00 (EN 17126:2018)
Period of analysis:	8. 11. 2023 - 13.11.2023
Test temperature:	20°C ± 1°C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 5344
Appearance of the product:	white powder
Product diluent:	hard water
Test concentration:	2 % 3 %
Contact time:	10 min
Interfering substances:	0,3 g/l BSA (clean conditions)
Test organisms:	<i>Bacillus subtilis</i> <i>Bacillus cereus</i> <i>Clostridium difficile</i> ribotype 027
Incubation conditions:	37 °C ± 1 °C, 24-48 hours 37 °C ± 1 °C, 5 days

ATCC 6633
DSM 106266
DSM 27147

1. Preparation of the test suspension
2. Preparation of product test solutions
3. Quantitative suspension test
4. Incubation and calculation
5. Expression and interpretation of results

Sporicidal activity – the capability of a product to produce a reduction in the number of bacterial spores belonging to reference strains of *Bacillus subtilis* and *Bacillus cereus* under defined conditions by at least a 4 lg reduction (10^4).
Sporicidal activity against *Clostridium difficile* – the capability of a product to produce a reduction in the number of bacterial spores belonging to reference strain of *Clostridium difficile* under defined conditions by at least a 4 lg reduction (10^4).

EN 17126:2018 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants in the medical area – Test method and requirements (phase 2, step 1), December 2018

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

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

Testing the efficacy of chemical disinfectant **OXISEPT** on *Bacillus subtilis* ATCC 6633

Test suspensions:

Dilution	Vc1	Vc2	lgN	lgN ₀
10 ⁻⁵	212	224		
10 ⁻⁶	21	22	7,34	6,34
$\Phi = 2,18 \times 10^7$			$7,17 \leq \lg N \leq 7,7$	$6,17 \leq \lg N_0 \leq 6,7$

Verification of methodology

Validation of suspension N _{vo}		Validation of suspension N _v		Neutralizer toxicity control (B)	
Vc1	52	Vc1	60	Vc1	40
Vc2	62	Vc2	58	Vc2	56
$30 \leq 57 \leq 160$		$59 \geq 0,5 N_{v0}$		$48 \geq 0,5 N_{v0}$	

Validation of selected experimental conditions (A)

Testing conditions	Vc1	Vc2	Φ_A
10 min, 0,3 g/l BSA (clean conditions), 20°C	70	50	$60 \geq 0,5 N_{v0}$

Method validation (C)

Testing conditions	Vc1	Vc2	Φ_C
3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	50	56	$53 \geq 0,5 N_{v0}$

Testing the efficacy of chemical disinfectant

Testing conditions	Dilution after test procedure	Vc1	Vc2	lgNa lgNa=lg($\Phi_A \times 10$)	lgR lgN ₀ =6,34
2 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	10 ⁰	<14	<14	<2,15	>=4,19
3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	10 ⁰	<14	<14	<2,15	>=4,19

Note: Vc = value is the number of cfu per ml, Φ = average Vc1 and Vc2 (1. + 2. duplicate Vc values), N = the number of cfu/ml of the bacterial test suspension, N₀ = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_v = the number of cfu/ml of the bacterial test suspension for validation N_{v0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_a = the number of surviving bacteria per ml in the test mixture, A, B, C = the number of surviving bacteria per ml in control tests (A – experimental conditions control, B – neutralizer toxicity validation, C – method validation), R = N₀/ N_a = the reduction in viability, or lg R = lg N₀ – lg N_a

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

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Testing the efficacy of chemical disinfectant **OXISEPT** on *Bacillus cereus* DSM 106266

Test suspensions:

Dilution	Vc1	Vc2	IgN	IgN ₀
10 ⁻⁵	>330	>330		
10 ⁻⁶	31	36	7,53	6,53
$\Phi = 3,35 \times 10^7$			$7,17 \leq \lg N \leq 7,7$	$6,17 \leq \lg N_0 \leq 6,7$

Verification of methodology

Validation of suspension N _{v0}		Validation of suspension N _{vb}		Neutralizer toxicity control (B)	
Vc1	72	Vc1	81	Vc1	73
Vc2	81	Vc2	76	Vc2	72
$30 \leq 76,5 \leq 160$		$78,5 \geq 0,5 N_{v0}$		$72,5 \geq 0,5 N_{v0}$	

Validation of selected experimental conditions (A)

Testing conditions	Vc1	Vc2	Φ_A
10 min, 0,3 g/l BSA (clean conditions), 20°C	67	84	$75,5 \geq 0,5 N_{v0}$

Method validation (C)

Testing conditions	Vc1	Vc2	Φ_C
3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	94	65	$79,5 \geq 0,5 N_{v0}$

Testing the efficacy of chemical disinfectant

Testing conditions	Dilution after test procedure	Vc1	Vc2	IgNa $\lg Na = \lg(\Phi_A \times 10)$	IgR $\lg N_0 = 6,53$
2 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	10 ⁰	76	90	2,92	3,61
3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	10 ⁰	<14	<14	<2,15	>=4,38

Note: Vc = value is the number of cfu per ml, Φ = average Vc1 and Vc2 (1. + 2. duplicate Vc values), N = the number of cfu/ml of the bacterial test suspension, N₀ = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_v = the number of cfu/ml of the bacterial test suspension for validation N_{v0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, Na = the number of surviving bacteria per ml in the test mixture, A, B, C = the number of surviving bacteria per ml in control tests (A – experimental conditions control, B – neutralizer toxicity validation, C – method validation), R = N₀ / Na = the reduction in viability, or $\lg R = \lg N_0 - \lg Na$

Description: Testing the efficacy of chemical disinfectants and antiseptics

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Testing the efficacy of chemical disinfectant **OXISEPT** on *Clostridium difficile* ribotype 027 DSM 27147

Test suspensions:

Dilution	Vc1	Vc2	lgN	lgN ₀
10 ⁻⁵	144	178		
10 ⁻⁶	14	19	7,21	6,21
$\Phi = 1,61 \times 10^7$			$7,17 \leq \lg N \leq 7,7$	$6,17 \leq \lg N_0 \leq 6,7$

Verification of methodology

Validation of suspension N _{vo}		Validation of suspension N _{vb}		Neutralizer toxicity control (B)	
Vc1	52	Vc1	34	Vc1	36
Vc2	44	Vc2	41	Vc2	44
$30 \leq 48 \leq 160$		$37,5 \geq 0,5 N_{vo}$		$40 \geq 0,5 N_{vo}$	

Validation of selected experimental conditions (A)

Testing conditions	Vc1	Vc2	Φ_A
10 min, 0,3 g/l BSA (clean conditions), 20°C	35	40	$37,5 \geq 0,5 N_{vo}$

Method validation (C)

Testing conditions	Vc1	Vc2	Φ_C
3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	48	55	$51,5 \geq 0,5 N_{vo}$

Testing the efficacy of chemical disinfectant

Testing conditions	Dilution after test procedure	Vc1	Vc2	lgNa lgNa=lg(ΦAx10)	lgR lgN ₀ =6,21
2 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	10 ⁰	30	36	2,52	3,69
3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	10 ⁰	14	16	2,18	4,03

Note: Vc = value is the number of cfu per ml, Φ = average Vc1 and Vc2 (1. + 2. duplicate Vc values), N = the number of cfu/ml of the bacterial test suspension, N₀ = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_v = the number of cfu/ml of the bacterial test suspension for validation N_{v0} = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, Na = the number of surviving bacteria per ml in the test mixture, A, B, C = the number of surviving bacteria per ml in control tests (A – experimental conditions control, B – neutralizer toxicity validation, C – method validation), R = N₀/ Na = the reduction in viability, or lg R = lg N₀ – lg Na

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Evaluation of SPORICIDAL (EN 17126:2018) activity of the product OXISEPT

Strain	Test conditions	lgR	lgR
<i>Bacillus subtilis</i> ATCC 6633	2 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	≥4	> 4
<i>Bacillus subtilis</i> ATCC 6633	3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	≥4	> 4
<i>Bacillus cereus</i> DSM 106266	2 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	≥4	< 4
<i>Bacillus cereus</i> DSM 106266	3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	≥4	> 4
<i>Clostridium difficile</i> ribotype 027 DSM 27147	2 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	≥4	< 4
<i>Clostridium difficile</i> ribotype 027 DSM 27147	3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C	≥4	> 4

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} and V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the bacterial test suspension, N_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, NV = the number of cfu/ml of the bacterial test suspension for validation NV_0 = the number of cfu/ml of the bacterial test suspension for validation in the test mixture, A, B, C at the beginning of the contact time = 0, N_a = the number of surviving bacteria per ml in the test mixture, A, B, C = the number of surviving bacteria per ml in control tests (A – experimental conditions control, B – neutralizer toxicity validation, C – method validation), $R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

Prepared by: Bc. Šárka Vašíčková Dohnalová, Lab Technican

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Conclusion:

The tested product: **OXISEPT**
Batch number: 184/09.2023
Standard: EN 17126:2018
Test method: dilution neutralization method

For conditions: 3 %, 10 min, 0,3 g/l BSA (clean conditions), 20°C
Bacillus subtilis, Bacillus cereus, Clostridium difficile ribotype 027
the efficacy is confirmed.

The tested product is capable of reducing the number of viable cells of the relevant organisms under defined conditions to the declared values, and consequently, can be called sporicidal .

Approved by: Ing. Barbora Stoklásková, Leader of Study

Hodonín, 22.11.2023

