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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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Test report No. S18-2/2020

DETERMINATION OF BACTERICIDAL (EN 13727:2012+A2:2015), YEASTICIDAL (EN 13624:2013) AND VIRUCIDAL (EN 14476:2013 +A2:2019) ACTIVITY OF THE PRODUCT **NOCOLYSE**

Sample ID: S18/2020
Sample name: **Nocolyse**
Client: REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania
Producer: REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania
Sampling point: REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania

Incoming date:
22.1.2020

Delivery date:
15.10.2020

Hodonín, 15.10.2020

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Ing. Jana Šlitrová, Head of Laboratory

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

Subject of testing:
Determination of bactericidal, yeasticidal and virucidal activity of the product.

Identification of the sample:

Name of the product:	**Nocolyse**
Batch number:	061219N/1
Date of manufacture:	06/12/2019
Expiry date:	12/2021
Manufacturer:	REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania
Incoming date:	22.1.2020
Storage conditions:	stated by the manufacturer
Active ingredients:	CAS 7722-84-1 hydrogen peroxide 6%

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13624:2013)
Period of analysis:	20.4. – 22.4.2020
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the product:	colourless liquid
Test concentration:	100% (concentrated)*
Contact time:	15 min
Interfering substances:	3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	*Candida albicans* ATCC 10231
Incubation conditions:	30 °C ± 1 °C, 48 hours and additional period of 24 or 48 hours

Test procedure:

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

Note:
Fungicidal activity – the capability of a product to produce a reduction in the number of viable fungi belonging to reference strains under defined conditions by at least a 4 lg reduction (10^4).
Yeasticidal activity – the capability of a product to produce a reduction in the number of viable yeast cells of relevant test organisms under defined conditions by at least a 4 lg reduction (10^4).
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

The standard:
EN 13624:2013 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area - Test method and requirements (phase 2, step 1) September 2013

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
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Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

The Number of CFU in the tested product: 0 CFU/ml

1. Testing the efficacy of chemical disinfectant **Nocolyse** on *Candida albicans* ATCC 10231

Tab No. 1.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	60	Φ_{Nvo}= 58.5	V_{c1}	33	Φ_A = 50.5	V_{c1}	40	Φ_B = 37.5	V_{c1}	37	Φ_C = 36.5
V_{c2}	57		V_{c2}	68		V_{c2}	35		V_{c2}	36	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes		x	yes		x	yes		x	yes	
	no			no			no			no	

Validation of suspension (N_{VB})	V_{c1}	43	V_{c2}	58	Φ_{NVB}	50.5	$30 \leq \Phi_{NVB}\ (N_{VB}/1000) \leq 160$	
							x yes	no

Tab No. 1.2 Test suspension

Test suspension N*	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)*
$\Phi = 50 \times 10^7 = \lg 8.70$	10^{-6}	> 330	> 330	$\lg N_0 = \lg N/100 = \lg 6.70$
$8.17 \leq \lg N \leq 8.70$	10^{-7}	57	43	$6.17 \leq \lg N_0 \leq 6.70$
				x yes no

Tab No. 1.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Candida albicans* ATCC 10231

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 6.70$)**
100 / 15 / dirty	10^0	< 14	< 14	< 2.15	**≥ 4.55**

2. Evaluation of yeasticidal activity of the product **Nocolyse**

Tab No. 2.1 The efficacy of chemical disinfectant **Nocolyse** on test strains – yeasticidal activity

Fungicidal activity of the product (EN 13624:2013)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]*	Interfering substances - conditions	lg R EN 13624:2013	**lg R**
Candida albicans ATCC 10231	20	15	100	dirty	≥ 4	> **4**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_{V0} = the number of cfu/ml of the test suspension for validation, N_a = the number of surviving fungi per ml in the test mixture, A, B, C = the number of surviving fungi per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), $R = N_0/ N_a$ = the reduction in viability, or $\lg R = \lg N_0 – \lg N_a$

* Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

Prepared by: Mgr. Karolína Světlíková, Lab Technician

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13727:2012+A2:2015)	
Period of analysis:	2.4. – 3.4.2020	
Test temperature:	20 °C ± 1 °C	
Test method:	dilution neutralization method	
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062	
Appearance of the product:	colourless liquid	
Test concentration:	100% (concentrated)*	
Contact time:	5 min, 15 min	
Interfering substances:	3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)	
Test organisms:	*Pseudomonas aeruginosa*	ATCC 15442
	Staphylococcus aureus	ATCC 6538
	Enterococcus hirae	ATCC 10541
	Escherichia coli K12	NCTC 10538
	Staphylococcus aureus	MRSA NRL/ST 13/760***
Incubation conditions:	37 °C ± 1 °C, 24 hours	

Test procedure:

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

Note:
Bactericidal activity – the capability of a product to produce a reduction in the number of viable bacterial cells of relevant organisms under defined conditions by at least a 5 lg reduction (10^5).
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.
*** Strain used according to client's request

The standard:
EN 13727:2012+A2:2015 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity in the medical area – Test method and requirements (phase 2, step 1) October 2015

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

3. Testing the efficacy of chemical disinfectant **Nocolyse** on *Pseudomonas aeruginosa* ATCC 15442

Tab No. 3.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	94	Φ_{Nvo}= 90	V_{c1}	107	Φ_A = 89.5	V_{c1}	76	Φ_B = 90	V_{c1}	55	Φ_C = 59
V_{c2}	86		V_{c2}	72		V_{c2}	104		V_{c2}	63	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	102	V_{c2}	86	Φ_{NVB}	94	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160		
							x	yes	no

Tab No. 3.2 Test suspension

Test suspension N*	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)*
$\Phi = 47 \times 10^8 = \lg 9.67$	10^{-7}	> 330	> 330	$\lg N_0 = \lg N/100 = \lg 7.67$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	53	41	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 3.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Pseudomonas aeruginosa* ATCC 15442

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.67$)**
100 / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 5.52**
100 / 15 / dirty	10^0	<14	<14	< 2.15	**≥ 5.52**

4. Testing the efficacy of chemical disinfectant **Nocolyse** on *Staphylococcus aureus* ATCC 6538

Tab No. 4.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	48	Φ_{Nvo}= 44	V_{c1}	52	Φ_A = 44.5	V_{c1}	40	Φ_B = 42.5	V_{c1}	36	Φ_C = 41.5
V_{c2}	40		V_{c2}	37		V_{c2}	45		V_{c2}	47	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	49	V_{c2}	40	Φ_{NVB}	44.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160		
							x	yes	no

Tab No. 4.2 Test suspension

Test suspension N*	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)*
$\Phi = 40.5 \times 10^8 = \lg 9.61$	10^{-7}	> 330	> 330	$\lg N_0 = \lg N/100 = \lg 7.61$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	42	39	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 4.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Staphylococcus aureus* ATCC 6538

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.61$)**
100 / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 5.46**
100 / 15 / dirty	10^0	<14	<14	< 2.15	**≥ 5.46**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a 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, N_V = the number of cfu/ml of the bacterial test suspension for validation, N_{V0} (A,C), N_{VB} (B) = the number of cfu/ml of the bacterial test suspensions 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 $\lg R = \lg N_0 – \lg N_a$

* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

5. Testing the efficacy of chemical disinfectant **Nocolyse** on *Enterococcus hirae* ATCC 10541

Tab No. 5.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	56	Φ_{Nvo}= 44	V_{c1}	37	Φ_A = 44	V_{c1}	43	Φ_B = 36.5	V_{c1}	24	Φ_C = 32.5
V_{c2}	32		V_{c2}	51		V_{c2}	30		V_{c2}	41	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	61	V_{c2}	38	Φ_{NVB}	49.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160		
							x	yes	no

Tab No. 5.2 Test suspension

Test suspension N*	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)*
$\Phi = 42 \times 10^8 = \lg 9.62$	10^{-7}	> 330	> 330	$\lg N_0 = \lg N/100 = \lg 7.62$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	53	31	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 5.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Enterococcus hirae* ATCC 10541

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R (lg N_0 = lg 7.62)**
100 / 5 / dirty	10^{-4}	55	43	6.69	**0.93**
100 / 15 / dirty	10^0	<14	<14	< 2.15	**≥ 5.47**

6. Testing the efficacy of chemical disinfectant **Nocolyse** on *Escherichia coli K12* NCTC 10538

Tab No. 6.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	57	Φ_{Nvo}= 45	V_{c1}	33	Φ_A = 39	V_{c1}	43	Φ_B = 34	V_{c1}	28	Φ_C = 34.5
V_{c2}	33		V_{c2}	45		V_{c2}	25		V_{c2}	41	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	49	V_{c2}	40	Φ_{NVB}	44.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160		
							x	yes	no

Tab No. 6.2 Test suspension

Test suspension N*	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)*
$\Phi = 44 \times 10^8 = \lg 9.64$	10^{-7}	> 330	> 330	$\lg N_0 = \lg N/100 = \lg 7.64$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	52	36	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 6.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Escherichia coli K12* NCTC 10538

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R (lg N_0 = lg 7.64)**
100 / 5 / dirty	10^0	<14	<14	< 2.15	**≥ 5.49**
100 / 15 / dirty	10^0	<14	<14	< 2.15	**≥ 5.49**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a 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, N_V = the number of cfu/ml of the bacterial test suspension for validation, N_{V0} (A,C), N_{VB} (B) = the number of cfu/ml of the bacterial test suspensions 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 $\lg R = \lg N_0 – \lg N_a$

* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

7. Testing the efficacy of chemical disinfectant **Nocolyse** on *Staphylococcus aureus* MRSA NRL/ST 13/760***

Tab No. 7.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})				Validation of selected experimental conditions (A)				Neutralizer toxicity control (B)				Method validation (C) Product conc.: 100%*			
V_{c1}	40	Φ_{Nvo}= 39.5		V_{c1}	33	Φ_A = 35		V_{c1}	37	Φ_B = 30.5		V_{c1}	38	Φ_C = 38.5	
V_{c2}	39			V_{c2}	37			V_{c2}	24			V_{c2}	39		
$30 \leq \Phi_{Nvo} \leq 160$				$\Phi_A \geq 0.5\ \Phi_{Nvo}$				$\Phi_B \geq 0.5\ \Phi_{Nvo}$				$\Phi_C \geq 0.5\ \Phi_{Nvo}$			
x	yes		no	x	yes		no	x	yes		no	x	yes		no

Validation of suspension (N_{VB})	V_{c1}	37	V_{c2}	41	Φ_{NVB}	39	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160			
							x	yes		no

Tab No. 7.2 Test suspension

Test suspension N*	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)*
$\Phi = 36 \times 10^8$ = lg 9.56	10^{-7}	> 330	> 330	lg N_0 = lg N/100 = lg 7.56
$9.17 \leq$ lg N ≤ 9.70	10^{-8}	34	38	$7.17 \leq$ lg $N_0 \leq 7.70$
				x yes no

Tab No. 7.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Staphylococcus aureus* MRSA NRL/ST 13/760***

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	lg N_a = lg (Φ_a x 10)	**lg R (lg N_0 = lg 7.56)**
100 / 5 / dirty	10^0	33	29	2.49	**5.07**
100 / 15 / dirty	10^0	<14	<14	< 2.15	**≥ 5.41**

8. Evaluation of bactericidal activity of the product **Nocolyse**

Tab No. 8.1 The efficacy of chemical disinfectant **Nocolyse** on test strains – bactericidal activity

Bactericidal activity of the product (EN 13727:2012+A2:2015)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]*	Interfering substances - conditions	lg R EN 13727:2012 +A2:2015	**lg R**
Pseudomonas aeruginosa ATCC 15442	20	5	100	dirty	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	5	100	dirty	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	5	100	dirty	≥ 5	**< 5**
Escherichia coli K12 NCTC 10538	20	5	100	dirty	≥ 5	**> 5**
Staphylococcus aureus MRSA NRL/ST 13/760***	20	5	100	dirty	≥ 5	**> 5**
Pseudomonas aeruginosa ATCC 15442	20	15	100	dirty	≥ 5	**> 5**
Staphylococcus aureus ATCC 6538	20	15	100	dirty	≥ 5	**> 5**
Enterococcus hirae ATCC 10541	20	15	100	dirty	≥ 5	**> 5**
Escherichia coli K12 NCTC 10538	20	15	100	dirty	≥ 5	**> 5**
Staphylococcus aureus MRSA NRL/ST 13/760***	20	15	100	dirty	≥ 5	**> 5**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a 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, N_V = the number of cfu/ml of the bacterial test suspension for validation, N_{V0} (A,C), N_{VB} (B) = the number of cfu/ml of the bacterial test suspensions 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

* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

*** Strain used according to client's request

Prepared by: Mgr. Karolína Světlíková, Lab Technician

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A2:2019)
Period of analysis:	29.7. – 7.8.2020
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Appearance of the product:	colourless liquid
Test concentration:	100% (concentrated)*/**
Contact time:	5 min, 15 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K51622203930, expiry date: 31.7.2021
Test virus:	*Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 (2nd passage)
Cell lines:	HeLa cells (24th passage)
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.
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.
** The test was performed by using MicroSpin™ S 400 HR (2 pcs).

The standard:
EN 14476:2013 +A2:2019 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 + July 2019

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

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

Tab No. 9.1 Table of results of product **Nocolyse** on *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5

Product	Concentration **	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 5 min	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
Nocolyse	100%*	clean	-	4.50	4.50	-	-
Nocolyse	100%*	dirty	4.50	4.50	4.50	-	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	-	7.00	6.17
			Virus titration, time = 0				
Virus control	-	PBS	10.00	-	-	10.00	10.00
Virus control	-	clean	10.00	10.00	10.00	-	-
Virus control	-	dirty	9.83	9.83	9.83	-	-

Tab No. 9.2 Testing the efficacy of chemical disinfectant **Nocolyse** 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	$\Delta\log_{10}$ $TCID_{50}$
100%*	10.00	clean	5 min	4.50	**5.50**
100%*	10.00	clean	15 min	4.50	**5.50**
100%*	9.83	dirty	5 min	4.50	**5.33**
100%*	9.83	dirty	15 min	4.50	**5.33**

Tab No. 9.3 Testing the efficacy of chemical disinfectant **Formaldehyde** 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	$\Delta\log_{10}$ $TCID_{50}$
0.7 % (w/v)	10.00	PBS	30 min	7.00	**3.00**
0.7 % (w/v)	10.00	PBS	60 min	6.17	**3.83**

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 97% (RTU) 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 (2 pcs).

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

10. Evaluation of virucidal activity of the product **Nocolyse**

Tab No. 10.1 The efficacy of chemical disinfectant **Nocolyse** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013 +A2:2019)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]**	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013 +A2:2019	$\Delta\log_{10}$ $TCID_{50}$
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	5	100*	clean	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	15	100*	clean	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	5	100*	dirty	≥ 4	**> 4**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	15	100*	dirty	≥ 4	**> 4**

Tab No. 10.1 The efficacy of chemical disinfectant **Formaldehyde** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013 +A2:2019)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013 +A2:2019	$\Delta\log_{10}$ $TCID_{50}$
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	30	0.7 % (w/v)	PBS	3.0 – 5.0	**3.00**
Adenovirus type 5, strain Adenoid 75, ATCC VR-5	20	60	0.7 % (w/v)	PBS	3.5 – 5.5	**3.83**

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 97% (RTU) 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 (2 pcs).

Prepared by: Bc. Iva Čížová, Lab Technician

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A2:2019)
Period of analysis:	4.6. – 12.6.2020
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Appearance of the product:	colourless liquid
Test concentration:	100% (concentrated)*/**
Contact time:	5 min, 15 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K51622203930, expiry date: 31.7.2021
Test virus:	*Murine norovirus (MNV)* strain S99, RVB-651 (3rd passage)
Cell lines:	RAW 264.7 *Murine macrophage* cell line (3rd passage)
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.
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.
** The test was performed by using MicroSpin™ S 400 HR (2 pcs).

The standard:
EN 14476:2013 +A2:2019 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 + July 2019

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

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

Tab No. 11.1 Table of results of product **Nocolyse** on *Murine norovirus (MNV)* strain S99, RVB-651

Product	Concentration **	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 5 min	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
Nocolyse	100%*	clean	-	4.50	4.50	-	-
Nocolyse	100%*	dirty	4.50	5.50	4.83	-	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	-	7.50	6.50
			Virus titration, time = 0				
Virus control	-	PBS	9.83	-	-	9.83	10.00
Virus control	-	clean	10.00	10.00	10.00	-	-
Virus control	-	dirty	9.83	10.00	10.00	-	-

Tab No. 11.2 Testing the efficacy of chemical disinfectant **Nocolyse** 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%*	10.00	clean	5 min	4.50	**5.50**
100%*	10.00	clean	15 min	4.50	**5.50**
100%*	9.83	dirty	5 min	5.50	**4.33**
100%*	9.83	dirty	15 min	4.83	**5.00**

Tab No. 11.3 Testing the efficacy of chemical disinfectant **Formaldehyde** 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	$\Delta\log_{10}$ $TCID_{50}$
0.7 % (w/v)	9.83	PBS	30 min	7.50	**2.33**
0.7 % (w/v)	9.83	PBS	60 min	6.50	**3.33**

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 97% (RTU) 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 (2 pcs).

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

12. Evaluation of virucidal activity of the product **Nocolyse**

Tab No. 12.1 The efficacy of chemical disinfectant **Nocolyse** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013 +A2:2019)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]**	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013 +A2:2019	$\Delta\log_{10}$ $TCID_{50}$
Murine norovirus (MNV) strain S99, RVB-651	20	5	100*	clean	≥ 4	**> 4**
Murine norovirus (MNV) strain S99, RVB-651	20	15	100*	clean	≥ 4	**> 4**
Murine norovirus (MNV) strain S99, RVB-651	20	5	100*	dirty	≥ 4	**> 4**
Murine norovirus (MNV) strain S99, RVB-651	20	15	100*	dirty	≥ 4	**> 4**

Tab No. 12.1 The efficacy of chemical disinfectant **Formaldehyde** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013 +A2:2019)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013 +A2:2019	$\Delta\log_{10}$ $TCID_{50}$
Murine norovirus (MNV) strain S99, RVB-651	20	30	0.7 % (w/v)	PBS	1.0 – 3.0	**2.33**
Murine norovirus (MNV) strain S99, RVB-651	20	60	0.7 % (w/v)	PBS	2.0 – 4.0	**3.33**

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 97% (RTU) 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 (2 pcs).

Prepared by: Bc. Iva Čížová, Lab Technician

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

Experiment conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 14476:2013 +A2:2019)
Period of analysis:	2.9. – 10.9.2020
Test temperature:	20 °C ± 1 °C
Method of titration:	virus titration on monolayers of cells on microtitre plates
Appearance of the product:	colourless liquid
Test concentration:	100% (concentrated)*/**
Contact time:	5 min, 15 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Reference product:	Formaldehyde 36 – 38% solution p.a., CAS: 50-00-0, Batch No: K51622203930, expiry date: 31.7.2021
Test virus:	*Poliovirus* type 1, LSc-2ab (2nd passage)
Cell lines:	HeLa cells (12th passage)
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.
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.
** The test was performed by using MicroSpin™ S 400 HR (2 pcs).

The standard:
EN 14476:2013 +A2:2019 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 + July 2019

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

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

Tab No. 13.1 Table of results of product **Nocolyse** on *Poliovirus* type 1, LSc-2ab

Product	Concentration **	Interfering substances	Level of cytoxicity	- $\log_{10}$ $TCID_{50}$ after 5 min	- $\log_{10}$ $TCID_{50}$ after 15 min	- $\log_{10}$ $TCID_{50}$ after 30 min	- $\log_{10}$ $TCID_{50}$ after 60 min
Nocolyse	100%*	clean	-	4.50	4.50	-	-
Nocolyse	100%*	dirty	4.50	5.00	4.50	-	-
Formaldehyde	0.7 % (w/v)	PBS	3.50	-	-	8.00	6.67
			Virus titration, time = 0				
Virus control	-	PBS	9.50	-	-	9.50	9.67
Virus control	-	clean	9.50	9.50	9.33	-	-
Virus control	-	dirty	9.33	9.33	9.33	-	-

Tab No. 13.2 Testing the efficacy of chemical disinfectant **Nocolyse** 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%*	9.50	clean	5 min	4.50	**5.00**
100%*	9.50	clean	15 min	4.50	**5.00**
100%*	9.33	dirty	5 min	5.00	**4.33**
100%*	9.33	dirty	15 min	4.50	**4.83**

Tab No. 13.3 Testing the efficacy of chemical disinfectant **Formaldehyde** 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	$\Delta\log_{10}$ $TCID_{50}$
0.7 % (w/v)	9.50	PBS	30 min	8.00	**1.50**
0.7 % (w/v)	9.50	PBS	60 min	6.67	**2.83**

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 97% (RTU) 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 (2 pcs).

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

14. Evaluation of virucidal activity of the product **Nocolyse**

Tab No. 14.1 The efficacy of chemical disinfectant **Nocolyse** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013 +A2:2019)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]**	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013 +A2:2019	$\Delta\log_{10}$ $TCID_{50}$
Poliovirus type 1, LSc-2ab	20	5	100*	clean	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	15	100*	clean	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	5	100*	dirty	≥ 4	**> 4**
Poliovirus type 1, LSc-2ab	20	15	100*	dirty	≥ 4	**> 4**

Tab No. 14.1 The efficacy of chemical disinfectant **Formaldehyde** on test viruses – virucidal activity

Virucidal activity of the product (EN 14476:2013 +A2:2019)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances - conditions	$\Delta\log_{10}$ $TCID_{50}$ EN 14476:2013 +A2:2019	$\Delta\log_{10}$ $TCID_{50}$
Poliovirus type 1, LSc-2ab	20	30	0.7 % (w/v)	PBS	0.5 – 2.5	**1.50**
Poliovirus type 1, LSc-2ab	20	60	0.7 % (w/v)	PBS	2.0 – 4.5	**2.83**

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 97% (RTU) 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 (2 pcs).

Prepared by: Ing. Eva Kremlová, Lab Technician
Bc. Iva Čížová, Lab Technician

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

Interpretation:
Results of tests are in Tabs.
According to EN 13727:2012+A2:2015 the tested concentrated* product **Nocolyse,** batch No. 061219N/1, in the contact time 15 min under dirty conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of viable bacterial cells of *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Escherichia coli K12* NCTC 10538 by at least a 5 lg reduction.

According to EN 13727:2012+A2:2015 the tested concentrated* product **Nocolyse,** batch No. 061219N/1, in the contact times 5 min and 15 min under dirty conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of viable bacterial cells of *Staphylococcus aureus* MRSA NRL/ST 13/760*** by at least a 5 lg reduction.

According to EN 13624:2013 the tested concentrated* product **Nocolyse,** batch No. 061219N/1, in the contact time 15 min under dirty conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of viable yeast cells of *Candida albicans* ATCC 10231 by at least a 4 lg reduction.

According to EN 14476:2013 +A2:2019 the tested concentrated*/** product **Nocolyse,** batch No. 061219N/1, in the contact times 5 min and 15 min under clean and dirty 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 *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5 particles under defined conditions by at least a 4 lg reduction.

According to EN 14476:2013 +A2:2019 the tested concentrated*/** product **Nocolyse,** batch No. 061219N/1, in the contact times 5 min and 15 min under clean and dirty 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 *Murine norovirus (MNV)* strain S99, RVB-651 particles under defined conditions by at least a 4 lg reduction.

According to EN 14476:2013 +A2:2019 the tested concentrated*/** product **Nocolyse,** batch No. 061219N/1, in the contact times 5 min and 15 min under clean and dirty 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 97% (RTU product) or less, as some dilution is always produced by adding the test organisms and interfering substance.
** The test was performed by using MicroSpinTM S 400 HR (2 pcs).
*** Strain used according to client's request

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 2.4. – 10.9.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

Conclusion:
The product **Nocolyse** is capable of reducing the number of viable bacterial cells of the relevant organisms under defined conditions (EN 13727:2012+A2:2015 – concentrated, 15 min, dirty conditions, 20 °C) to the declared values, and consequently, can be called bactericidal.
The product **Nocolyse** is capable of reducing the number of viable bacterial cells of *Staphylococcus aureus* MRSA under defined conditions (EN 13727:2012+A2:2015 – concentrated, 15 min, dirty conditions, 20 °C) to the declared values, and consequently, can be called bactericidal on *Staphylococcus aureus* MRSA.
The product **Nocolyse** is capable of reducing the number of viable yeast cells of the relevant organisms under defined conditions (EN 13624:2013 – concentrated, 15 min, dirty conditions, 20 °C) to the declared values, and consequently, can be called yeasticidal.
The product **Nocolyse** is capable of reducing the number of infectious *Adenovirus*, *Murine norovirus, Poliovirus* particles under defined conditions (EN 14476:2013 +A2:2019, *Adenovirus*, *Murine norovirus, Poliovirus* – concentrated, 5 minand 15 min, clean and dirty conditions, 20 °C) to the declared values, and consequently, can be called virucidal.

15.10.2020, Hodonín

Approved by: Ing. Barbora Stoklásková

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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. S18/2020

DETERMINATION OF FUNGICIDAL (EN 13624:2013) ACTIVITY OF THE PRODUCT **NOCOLYSE**

Sample ID: S18/2020
Sample name: **Nocolyse**
Client: REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania
Producer: REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania
Sampling point: REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania

Incoming date:
22.1.2020

Delivery date:
12.3.2020

Hodonín, 12.3.2020

TESTING LABORATORY CERTIFIED BY CZECH ACCREDITATION INSTITUTE
Chemila, spol. s.r.o.
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Ing. Jana Šlitrová, Head of Laboratory

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 19.2. – 22.2.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

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

Identification of the sample:

Name of the product:	**Nocolyse**
Batch number:	061219N/1
Date of manufacture:	06/12/2019
Expiry date:	12/2021
Manufacturer:	REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania
Incoming date:	22.1.2020
Storage conditions:	stated by the manufacturer
Active ingredients:	CAS 7722-84-1 hydrogen peroxide 6%

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13624:2013)
Period of analysis:	19.2. – 22.2.2020
Test temperature:	20 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the product:	colourless liquid
Test concentration:	100% (concentrated)*
Contact time:	15 min, 30 min, 60 min
Interfering substances:	3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	*Aspergillus brasiliensis (niger)* ATCC 16404
Incubation conditions:	30 °C ± 1 °C, 48 hours and additional period of 24 or 48 hours

Test procedure:

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

Note:
Presence of a high concentration (at least 75%) of *Aspergillus brasiliensis* spiny spores in the test suspension – yes.
Fungicidal activity – the capability of a product to produce a reduction in the number of viable fungi belonging to reference strains under defined conditions by at least a 4 lg reduction (10^4).
Yeasticidal activity – the capability of a product to produce a reduction in the number of viable yeast cells of relevant test organisms under defined conditions by at least a 4 lg reduction (10^4).
$R = N_0 / N_a$ = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$
* Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

The standard:
EN 13624:2013 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area - Test method and requirements (phase 2, step 1) September 2013

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 19.2. – 22.2.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

The Number of CFU in the tested product: 0 CFU/ml

1. Testing the efficacy of chemical disinfectant **Nocolyse** on *Aspergillus brasiliensis (niger)* ATCC 16404

Tab No. 1.1 Verification of methodology, dirty conditions

Validation of suspension (N_{V0})			Validation of selected experimental conditions (A)			Neutralizer toxicity control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	36	Φ_{Nvo}= 38.5	V_{c1}	50	Φ_A = 38.5	V_{c1}	41	Φ_B = 37	V_{c1}	38	Φ_C = 37
V_{c2}	41		V_{c2}	27		V_{c2}	33		V_{c2}	36	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Validation of suspension (N_{VB})	V_{c1}	33	V_{c2}	48	Φ_{NVB}	40.5	$30 \leq \Phi_{NVB}$ (N_{VB}/1000) $\leq$ 160			
							x	yes		no

Tab No. 1.2 Test suspension

Test suspension N* $\Phi = 35.5 \times 10^7 = \lg 8.55$ $8.17 \leq \lg N \leq 8.70$	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)* $\lg N_0 = \lg N/100 = \lg 6.55$ $6.17 \leq \lg N_0 \leq 6.70$			
	10^{-6}	> 165	> 165				
	10^{-7}	35	36				
				x	yes		no

Tab No. 1.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Aspergillus brasiliensis (niger)* ATCC 16404

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 6.55$)**
100 / 15 / dirty	10^0	< 14	< 14	< 2.15	**≥ 4.40**
100 / 30 / dirty	10^0	< 14	< 14	< 2.15	**≥ 4.40**
100 / 60 / dirty	10^0	< 14	< 14	< 2.15	**≥ 4.40**

2. Evaluation of fungicidal activity of the product **Nocolyse**

Tab No. 2.1 The efficacy of chemical disinfectant **Nocolyse** on test strains – fungicidal activity

Fungicidal activity of the product (EN 13624:2013)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]*	Interfering substances - conditions	lg R EN 13624:2013	**lg R**
Aspergillus brasiliensis (niger) ATCC 16404	20	15	100	dirty	≥ 4	**> 4**
Aspergillus brasiliensis (niger) ATCC 16404	20	30	100	dirty	≥ 4	**> 4**
Aspergillus brasiliensis (niger) ATCC 16404	20	60	100	dirty	≥ 4	**> 4**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_{V0} = the number of cfu/ml of the test suspension for validation, N_a = the number of surviving fungi per ml in the test mixture, A, B, C = the number of surviving fungi per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation), R = N_0/ N_a = the reduction in viability, or $\lg R = \lg N_0 - \lg N_a$

* Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

Prepared by: Ing. Barbora Stoklásková, Lab Technician

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 19.2. – 22.2.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

Interpretation:
Results of tests are in Tabs.
According to EN 13624:2013 the tested concentrated* product **Nocolyse,** batch No. 061219N/1, in the contact times 15 min, 30 min and 60 min under dirty conditions at temperature 20 °C ± 1 °C by the dilution neutralization method **decreased** the number of mould spores of *Aspergillus brasiliensis (niger)* ATCC 16404 by at least a 4 lg reduction.
* Product can only be tested at a concentration of 97% (RTU) or less, as some dilution is always produced by adding the test organisms and interfering substance.

Conclusion:
The product **Nocolyse** is capable of reducing the number of mould spores of the relevant organisms under defined conditions (EN 13624:2013 – concentrated, 15 min, 30 min, 60 min, dirty, 20 °C) to the declared values, and consequently, can be called fungicidal on *Aspergillus brasiliensis (niger).*

12.3.2020, Hodonín

Chemila, spol. s.r.o.
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Ing. Eva Kremlová, Leader of Study

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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.

Copy No.: 1
Issue No.: 1

Test report No. S374/2020

DETERMINATION OF BACTERICIDAL (EN 13697:2015+A1:2019), FUNGICIDAL (EN 13697:2015+A1:2019), MYCOBACTERICIDAL, TUBERCULOCIDAL (EN 14348:2005) AND SPORICIDAL (EN 13704:2018) ACTIVITY OF THE PRODUCT **NOCOLYSE**

Sample ID: S374/2020
Sample name: **Nocolyse**
Client: REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania
Producer: OXY'PHARM SARL, 829 rue Marcel Paul 94500 Champigny sur Marne, France
Sampling point: REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania

Incoming date:
10.8.2020

Delivery date:
15.12.2020

Hodonín, 15.12.2020

Ing. Jana Šlitrová, Head of Laboratory

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

Subject of testing:
Determination of bactericidal, fungicidal, mycobactericidal, tuberculocidal and sporicidal activity of the product.

Identification of the sample:

Name of the product:	**Nocolyse**
Batch number:	A220720N/3
Date of manufacture:	07/2020
Expiry date:	07/2022
Manufacturer:	OXY'PHARM SARL, 829 rue Marcel Paul 94500 Champigny sur Marne, France
Incoming date:	10.8.2020
Storage conditions:	stated by the manufacturer
Active ingredients:	CAS 7722-84-1 hydrogen peroxide 6%

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers** SOP-M-22-12 (EN 13697:2015+A1:2019)
Period of analysis:	1.12. – 2.12.2020
Test temperature:	18 °C ± 1 °C to 25 °C ± 1 °C
Test method:	dilution neutralization method
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062
Appearance of the product:	colourless liquid
Test concentration:	100% (concentrated)
Contact time:	15 min and 60 min
Interfering substances:	3.0 g/l BSA (dirty conditions)
Test organisms:	*Enterococcus hirae* ATCC 10541
Incubation conditions:	37 °C ± 1 °C, 24 hours

Test procedure:

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

Note:
Bactericidal activity – the capability of a product to produce a reduction in the number of viable bacterial cells of relevant organisms on carriers under defined conditions by at least a 4 lg reduction (10^4).
The drying time: 25 – 50 min

The standard:
EN 13697:2015+A1:2019 Chemical disinfectants and antiseptics – Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas – Test method and requirements without mechanical action (phase 2, step 2) April 2015 + August 2019

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

The Number of CFU in the tested product: 0 CFU/ml

1. Testing the efficacy of chemical disinfectant **Nocolyse** on carriers – bactericidal activity

Tab No. 1.1 Verification of methodology, dirty conditions

Test organisms	Test suspension N	Validation test	
		NT (Product conc.: 100%) Neutralization test	NC Neutralization control
Enterococcus hirae ATCC 10541	10^{-6}: 203, 208 10^{-7}: 19, 20 N: 6.71	10^{-3}: >330, >330 10^{-4}: 48, 32 NT: 6.60	10^{-3}: >330, >330 10^{-4}: 35, 41 NC: 6.58
Limit	$6.57 \leq \lg N \leq 7.10$	NT - Nc $\leq \pm 0.3$ lg	NC - Nc $\leq \pm 0.3$ lg

$N = \log_{10}[\{0.025 \bullet (x + x')\} /2 \bullet d]$ where x and x' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account
NC or $NT = \log_{10}[\{10 \bullet (y + y')\} /2 \bullet d]$
where y and y´ are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

Tab No. 1.2 Testing the efficacy of chemical disinfectant **Nocolyse** on test strain, dirty conditions, 15 min

Test organisms	Water control Nc	Test procedure Nd at concentrations / contact time (min)
		100% / 15
Enterococcus hirae ATCC 10541	10^{-3}: 196, 221 10^{-4}: 20, 22 Nc: 6.32 Nts: >100	10^{0}: <14, <14 Nd : < 2.15 Nts: 0 **R : ≥ 4.17**
Limit	Nts: <100 CFU/ml for active concentration	

$Nc \text{ or } Nd = \log_{10}[\{10 \bullet (a + a')\} /2 \bullet d]$
where a and a' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account
Reduction R = Nc – Nd

Tab No. 1.3 Testing the efficacy of chemical disinfectant **Nocolyse** on test strain, dirty conditions, 60 min

Test organisms	Water control Nc	Test procedure Nd at concentrations / contact time (min)
		100% / 60
Enterococcus hirae ATCC 10541	10^{-3}: 164, 239 10^{-4}: 18, 24 Nc: 6.31 Nts: >100	10^{0}: <14, <14 Nd : < 2.15 Nts: 0 **R : ≥ 4.16**
Limit	Nts: <100 CFU/ml for active concentration	

$Nc \text{ or } Nd = \log_{10}[\{10 \bullet (a + a')\} /2 \bullet d]$
where a and a' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account
Reduction R = Nc – Nd

2. Evaluation of bactericidal activity of the product **Nocolyse** on carriers

Tab No. 2.1 The efficacy of chemical disinfectant **Nocolyse** on test strains – bactericidal activity on carriers

Bactericidal and fungicidal activity of the product on carriers (EN 13697:2015+A1:2019)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	R EN 13697:2015+ A1:2019	**R**
Enterococcus hirae ATCC 10541	18-25	15	100	dirty	≥ 4	**> 4**
Enterococcus hirae ATCC 10541	18-25	60	100	dirty	≥ 4	**> 4**

Reduction R= Nc – Nd

Prepared by: Ing. Barbora Stoklásková, Lab Technician

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers** SOP-M-22-12 (EN 13697:2015+A1:2019)	
Period of analysis:	4.11. – 7.11.2020	
Test temperature:	18 °C ± 1 °C to 25 °C ± 1 °C	
Test method:	dilution neutralization method	
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062	
Appearance of the product:	colourless liquid	
Test concentration:	100% (concentrated)	
Contact time:	15 min, 60 min	
Interfering substances:	3.0 g/l BSA (dirty conditions)	
Test organisms:	*Candida albicans*	ATCC 10231
	Aspergillus brasiliensis (niger)	ATCC 16404
Incubation conditions:	30 °C ± 1 °C, 48 hours and additional period of 24 or 48 hours	

Test procedure:

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

Note:
Presence of a high concentration (at least 75%) of *Aspergillus brasiliensis* spiny spores in the test suspension – yes.
Fungicidal activity – the capability of a product to produce a reduction in the number of viable fungi of relevant organisms on carriers under defined conditions by at least 3 orders (10^3).
Yeasticidal activity – the capability of a product to produce a reduction in the number of viable fungi belonging to reference strain *Candida albicans* on carriers under defined conditions by at least a 3 lg reduction (10^3).
The drying time: 30-35 min.

The standard:
EN 13697:2015+A1:2019 Chemical disinfectants and antiseptics – Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas – Test method and requirements without mechanical action (phase 2, step 2) April 2015 + August 2019

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

3. Testing the efficacy of chemical disinfectant **Nocolyse** on carriers – fungicidal activity

Tab No. 3.1 Verification of methodology, dirty conditions

Test organisms	Test suspension N	Validation test	
		NT (Product conc.: 100%) Neutralization test	NC Neutralization control
Candida albicans ATCC 10231	10^{-5} : 173, 183 10^{-6} : 19, 16 N : 5.65	10^{-2} : >330, >330 10^{-3} : 74, 38 NT : 5.75	10^{-2} : >330, >330 10^{-3} : 50, 43 NC : 5.67
Aspergillus brasiliensis (niger) ATCC 16404	10^{-5} : >165, >165 10^{-6} : 26, 30 N : 5.85	10^{-2} : >165, >165 10^{-3} : 69, 81 NT : 5.88	10^{-2} : >165, >165 10^{-3} : 72, 71 NC : 5.85
Limit	$5.57 \leq \lg N \leq 6.10$	NT - Nc $\leq \pm$ 0.3 lg	NC - Nc $\leq \pm$ 0.3 lg

$N = \log_{10}[\{0.025 \bullet (x + x')\} /2 \bullet d]$
where x and x' are paired values for which the mean of the value falls between 14 and 330 colonies for yeast and 14 and 165 colonies for mould, d is the dilution factor for the dilution taken into account
$NC \text{ or } NT = \log_{10}[\{10 \bullet (y + y')\} /2 \bullet d]$
where y and y´ are paired values for which the mean of the value falls between 14 and 330 colonies for yeast and 14 and 165 colonies for mould, d is the dilution factor for the dilution taken into account

Tab No. 3.2 Testing the efficacy of chemical disinfectant **Nocolyse** on test strain, dirty conditions

Test organisms	Water control Nc	Test procedure Nd at concentrations / contact time (min)
		100% / 15
Candida albicans ATCC 10231	10^{-2} : >330, >330 10^{-3} : 43, 55 Nc : 5.69 Nts: >100	10^{0} : <14, <14 Nd : < 2.15 Nts: 0 **R : ≥ 3.54**
Aspergillus brasiliensis (niger) ATCC 16404	10^{-2} : >165, >165 10^{-3} : 92, 59 Nc : 5.88 Nts: >100	10^{0} : 21, 33 Nd : 2.43 Nts: 11 **R : 3.45**
Limit	Nts: <100 CFU/ml for active concentration	

$Nc \text{ or } Nd = \log_{10}[\{10 \bullet (a + a')\} /2 \bullet d]$
where a and a' are paired values for which the mean of the value falls between 14 and 330 colonies for yeast and 14 and 165 colonies for mould, d is the dilution factor for the dilution taken into account
Reduction R= Nc – Nd

Tab No. 3.2 Testing the efficacy of chemical disinfectant **Nocolyse** on test strain, dirty conditions

Test organisms	Water control Nc	Test procedure Nd at concentrations / contact time (min)
		100% / 60
Candida albicans ATCC 10231	10^{-2} : >330, >330 10^{-3} : 23, 49 Nc : 5.56 Nts: >100	10^{0} : <14, <14 Nd : < 2.15 Nts: 0 **R : ≥ 3.41**
Aspergillus brasiliensis (niger) ATCC 16404	10^{-2} : >165, >165 10^{-3} : 84, 47 Nc : 5.82 Nts: >100	10^{0} : <14, <14 Nd : < 2.15 Nts: 0 **R : ≥ 3.67**
Limit	Nts: <100 CFU/ml for active concentration	

$Nc \text{ or } Nd = \log_{10}[\{10 \bullet (a + a')\} /2 \bullet d]$
where a and a' are paired values for which the mean of the value falls between 14 and 330 colonies for yeast and 14 and 165 colonies for mould, d is the dilution factor for the dilution taken into account
Reduction R= Nc – Nd

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

4. Evaluation of fungicidal activity of the product **Nocolyse** on carriers

Tab No. 4.1 The efficacy of chemical disinfectant **Nocolyse** on test strains – fungicidal activity on carriers

Fungicidal activity of the product on carriers (EN 13697:2015+A1:2019)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	R EN 13697:2015+ A1:2019	**R**
Candida albicans ATCC 10231	18-25	15	100	dirty	≥ 3	**> 3**
Aspergillus brasiliensis (niger) ATCC 16404	18-25	15	100	dirty	≥ 3	**> 3**
Candida albicans ATCC 10231	18-25	60	100	dirty	≥ 3	**> 3**
Aspergillus brasiliensis (niger) ATCC 16404	18-25	60	100	dirty	≥ 3	**> 3**

Reduction R= Nc – Nd

Prepared by: Ing. Barbora Stoklásková, Lab Technician

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method**
	SOP-M-19-00 (EN 14348:2005)
Period of analysis:	10.11. – 1.12.2020
Test temperature:	20 °C ± 1 °C
Test method:	membrane filtration method
Filtration diluent:	rinsing liquid
Appearance of the product:	colourless liquid
Test concentration:	100% (concentrated)*
Contact time:	60 min
Interfering substances:	0.3 g/l BSA (clean conditions)
	3 g/l BSA and 3 ml/l sheep erythrocytes (dirty conditions)
Test organisms:	*Mycobacterium avium* ATCC 15769
	Mycobacterium terrae ATCC 15755
Incubation conditions:	37 °C ± 1 °C, 21 days

Test procedure:

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

Note:
Mycobactericidal activity – the capability of a product to produce a reduction in the number of viable cells of *Mycobacterium terrae* and *Mycobacterium avium* under defined conditions by at least a 4 lg reduction (10^4).
Tuberculocidal activity - the capability of a product to produce a reduction in the number of viable cells of *Mycobacterium terrae* under defined conditions by at least a 4 lg reduction (10^4).
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

The standard:
EN 14348:2005 Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test method and requirements (phase 2, step 1) January 2005

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

5. Testing the efficacy of chemical disinfectant **Nocolyse** on *Mycobacterium avium* ATCC 15769

Tab No. 5.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Membrane filtration control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	46	Φ_{Nvo} = 45.5	V_{c1}	42	Φ_A = 40.5	V_{c1}	35	Φ_B = 37.5	V_{c1}	38	Φ_C = 45
V_{c2}	45		V_{c2}	39		V_{c2}	40		V_{c2}	52	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 5.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Membrane filtration control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	46	Φ_{Nvo} = 45.5	V_{c1}	41	Φ_A = 44.5	V_{c1}	35	Φ_B = 37.5	V_{c1}	30	Φ_C = 43
V_{c2}	45		V_{c2}	48		V_{c2}	40		V_{c2}	56	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 5.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)*
$\Phi = 47.5 \times 10^8 = \lg 9.68$	10^{-7}	>165	>165	$\lg N_0 = \lg N/100 = \lg 7.68$
$9.17 \leq \lg N \leq 9.70$	10^{-8}	44	51	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 5.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Mycobacterium avium* ATCC 15769

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = \lg 7.68$)**
100 / 60 / clean	10^{-1}	<14	<14	< 3.15	**≥ 4.53**
100 / 60 / dirty	10^{-1}	<14	<14	< 3.15	**≥ 4.53**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0“), N_a = the number of viable cells per ml in the test mixture at the end of the contact time and before the membrane filtration, N_v = the number of cfu/ml of the test suspension for validation, N_{v0}= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0“), A,B,C = the number of viable cells per ml in control tests (A – experimental conditions control, B – membrane filtration validation, C – method validation), $R = N_0 / N_a$ or $\lg R = \lg N_0 - \lg N_a$ the reduction in viability
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

6. Testing the efficacy of chemical disinfectant **Nocolyse** on *Mycobacterium terrae* ATCC 15755

Tab No. 6.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Membrane filtration control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	46	Φ_{Nvo} = 45	V_{c1}	39	Φ_A = 39.5	V_{c1}	50	Φ_B = 41.5	V_{c1}	43	Φ_C = 39.5
V_{c2}	44		V_{c2}	40		V_{c2}	33		V_{c2}	36	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 6.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{vo})			Validation of selected experimental conditions (A)			Membrane filtration control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	46	Φ_{Nvo} = 45	V_{c1}	50	Φ_A = 41	V_{c1}	50	Φ_B = 41.5	V_{c1}	35	Φ_C = 37.5
V_{c2}	44		V_{c2}	32		V_{c2}	33		V_{c2}	40	
$30 \leq \Phi_{Nvo} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nvo}$			$\Phi_B \geq 0.5\ \Phi_{Nvo}$			$\Phi_C \geq 0.5\ \Phi_{Nvo}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 6.2 Test suspensions

Test suspension N	N	V_{c1}	V_{c1}	Test suspension N_0 (time = 0)*
$\Phi = 45 \times 10^8$ = lg 9.65	10^{-7}	>165	>165	lg N_0 = lg N/100 = lg 7.65
$9.17 \leq \lg N \leq 9.70$	10^{-8}	52	38	$7.17 \leq \lg N_0 \leq 7.70$
				x yes no

Tab No. 6.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Mycobacterium terrae* ATCC 15755

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	lg N_a = lg (Φ_a x 10)	**lg R (lg N_0 = lg 7.65)**
100 / 60 / clean	10^{-1}	15	17	3.21	**4.44**
100 / 60 / dirty	10^{-1}	27	21	3.38	**4.27**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0“), N_a = the number of viable cells per ml in the test mixture at the end of the contact time and before the membrane filtration, N_v = the number of cfu/ml of the test suspension for validation, N_{v0}= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0“), A,B,C = the number of viable cells per ml in control tests (A – experimental conditions control, B – membrane filtration validation, C – method validation), $R = N_0 / N_a$ or $\lg R = \lg N_0 - \lg N_a$ the reduction in viability
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

7. Evaluation of mycobactericidal and tuberculocidal activity of the product **Nocolyse**

Tab No. 7.1 The efficacy of chemical disinfectant **Nocolyse** on test strain – mycobactericidal and tuberculocidal activity

Mycobactericidal and tuberculocidal activity of the product (EN 14348:2005)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]*	Interfering substances - conditions	lg R EN 14348:2005	**lg R**
Mycobacterium avium ATCC 15769	20	60	100	clean	≥ 4	**> 4**
Mycobacterium terrae ATCC 15755	20	60	100	clean	≥ 4	**> 4**
Mycobacterium avium ATCC 15769	20	60	100	dirty	≥ 4	**> 4**
Mycobacterium terrae ATCC 15755	20	60	100	dirty	≥ 4	**> 4**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the test suspension, N_0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0“), N_a = the number of viable cells per ml in the test mixture at the end of the contact time and before the membrane filtration, N_v = the number of cfu/ml of the test suspension for validation, N_{v0}= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0“), A,B,C = the number of viable cells per ml in control tests (A – experimental conditions control, B – membrane filtration validation, C – method validation), $R = N_0 / N_a$ or $\lg R = \lg N_0 – \lg N_a$ the reduction in viability
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

Prepared by: Ing. Eva Kremlová, Lab Technician

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method** SOP-M-19-00 (EN 13704:2018)
Period of analysis:	21.9. – 24.9.2020
Test temperature:	20 °C ± 1 °C
Test method:	membrane filtration method
Filtration diluent:	rinsing liquid
Appearance of the product:	colourless liquid
Test concentration:	100% (concentrated)*
Contact time:	60 min
Interfering substances:	0.3 g/l BSA (clean conditions) 3.0 g/l BSA (dirty conditions)
Test organisms:	*Clostridium sporogenes* ATCC 19404
Incubation conditions:	30 °C ± 1 °C, minimum 3 and maximum 7 days

Test procedure:

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 the results

Note:
Sporicidal activity – the capability of a product to produce a reduction in the number of bacterial spores belonging to reference strain of *Bacillus subtilis* under defined conditions by at least a 3 lg reduction (10^3).
For specific applications aditional strain may be chosen, for example *Bacillus cereus* (ATCC 12826), *Clostridium sporogenes* (ATCC 19404).
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

The standard:
EN 13704:2018 Chemical disinfectants – Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2, step 1) July 2018

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

8. Testing the efficacy of chemical disinfectant **Nocolyse** on *Clostridium sporogenes* ATCC 19404

Tab No. 8.1.1 Verification of methodology, clean conditions

Validation of suspension (N_{v0})			Validation of selected experimental conditions (A)			Membrane filtration control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	37	Φ_{Nv} = 31	V_{c1}	26	Φ_A = 27.5	V_{c1}	30	Φ_B = 31.5	V_{c1}	28	Φ_C = 29
V_{c2}	25		V_{c2}	29		V_{c2}	33		V_{c2}	30	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 8.1.2 Verification of methodology, dirty conditions

Validation of suspension (N_{v0})			Validation of selected experimental conditions (A)			Membrane filtration control (B)			Method validation (C) Product conc.: 100%*		
V_{c1}	37	Φ_{Nv} = 31	V_{c1}	35	Φ_A = 28	V_{c1}	30	Φ_B = 31.5	V_{c1}	22	Φ_C = 30
V_{c2}	25		V_{c2}	21		V_{c2}	33		V_{c2}	38	
$30 \leq \Phi_{Nv0} \leq 160$			$\Phi_A \geq 0.5\ \Phi_{Nv0}$			$\Phi_B \geq 0.5\ \Phi_{Nv0}$			$\Phi_C \geq 0.5\ \Phi_{Nv0}$		
x	yes	no	x	yes	no	x	yes	no	x	yes	no

Tab No. 8.2 Test suspension

Test suspension N*	N	V_{c1}	V_{c1}	Test suspension N_0*
$\Phi = 36 \times 10^6 = \lg 7.56$	10^{-5}	>330	>330	$\lg N_0 = \lg N/100 = \lg 5.56$
$7.17 \leq \lg N \leq 7.70$	10^{-6}	41	31	$5.17 \leq \lg N_0 \leq 5.70$
				x yes / No

Tab No. 8.3 Testing the efficacy of chemical disinfectant **Nocolyse** on *Clostridium sporogenes* ATCC 19404

Test concentration (%)*/contact time (min)/conditions	Dilution after test procedure	V_{c1}	V_{c2}	$\lg N_a = \lg (\Phi_a \times 10)$	**lg R ($\lg N_0 = 5.56$)**
100 / 60 / clean	10^0	<14	<14	< 2.15	**≥ 3.41**
100 / 60 / dirty	10^0	<14	<14	< 2.15	**≥ 3.41**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the spore test suspension, N_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_a = the number of viable cells per ml in the test mixture at the end of the contact time and before the membrane filtration, N_{v0} = the number of cfu/ml of the spore validation test suspension, A,B,C = the number of surviving spores per ml in control tests (A – experimental conditions control, B – membrane filtration validation, C – method validation), $\lg R = \lg N_0 - \lg N_a$ = the reduction in viability

* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

9. Evaluation of sporicidal activity of the product **Nocolyse**

Tab No. 9.1 The efficacy of chemical disinfectant **Nocolyse** on test strains – sporicidal activity

Sporicidal activity of the product (EN 13704:2018)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]*	Interfering substances - conditions	lg R EN 13704:2018	**lg R**
Clostridium sporogenes ATCC 19404	20	60	100	clean	≥ 3	**> 3**
Clostridium sporogenes ATCC 19404	20	60	100	dirty	≥ 3	**> 3**

Note: V_c = value is the number of cfu per ml, Φ = average V_{c1} a V_{c2} (1. + 2. duplicate V_c values), N = the number of cfu/ml of the spore test suspension, N_0 = the number of cfu/ml of the bacterial test suspension at the beginning of the contact time = 0, N_a = the number of viable cells per ml in the test mixture at the end of the contact time and before the membrane filtration, N_{v0} = the number of cfu/ml of the spore validation test suspension, A,B,C = the number of surviving spores per ml in control tests (A – experimental conditions control, B – membrane filtration validation, C – method validation), lg R = lg N_0 – lg N_a = the reduction in viability
* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

Prepared by: Ing. Eva Kremlová, Lab Technician

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

Sample ID: S374/2020
Rep No: 254
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: REMIX COM SRL, Bucharest, Romania

Sampling date: 7.7.2020
Sample delivered: 10.8.2020
Testing date: 31.9. – 2.12.2020
Delivered amount: 10 x 1 l
Batch No: A220720N/3

Interpretation:
Results of tests are in Tabs.
According to EN 13697:2015+A1:2019 the tested concentrated product **Nocolyse,** batch No. A220720N/3, in the contact time 15 min and 60 min under dirty conditions at temperature 18 °C ± 1 °C to 25 °C ± 1 °C by the dilution neutralization method **decreased** on carriers (stainless steel discs) the number of viable bacterial cells of *Enterococcus hirae* ATCC 10541 by at least a 4 lg reduction.

According to EN 13697:2015+A1:2019 the tested concentrated product **Nocolyse,** batch No. A220720N/3, in the contact time 15 min and 60 min under dirty conditions at temperature 18 °C ± 1 °C to 25 °C ± 1 °C by the dilution neutralization method **decreased** on carriers (stainless steel discs) the number of viable yeast cells of *Candida albicans* ATCC 10231 and the number of mould spores of *Aspergillus brasiliensis (niger)* ATCC 16404 by at least a 3 lg reduction.

According to EN 14348:2005 the tested concentrated* product **Nocolyse,** batch No. A220720N/3, in the contact time 60 min under clean and dirty conditions at temperature 20 °C ± 1 °C by the membrane filtration method **decreased** the number of viable mycobacterial cells of *Mycobacterium avium* ATCC 15769 and *Mycobacterium terrae* ATCC 15755 by at least a 4 lg reduction.

According to EN 13704:2018 the tested concentrated* product **Nocolyse,** batch No. A220720N/3, in the contact time 60 min under clean and dirty conditions at temperature 20 °C ± 1 °C by the membrane filtration method **decreased** the number of viable bacterial spoores of *Clostridium sporogenes* ATCC 19404 by at least a 3 lg reduction.

* The product can only be tested at a concentration of 97% (RTU product) or less, as some dilution is always produced by adding the inoculum and interfering substance.

Conclusion:
The product **Nocolyse** is capable of reducing the number of viable bacterial cells of the relevant organism *Enterococcus hirae* on carriers under defined conditions (EN 13697:2015+A1:2019 – *Enterococcus hirae*, concentrated, 15 min and 60 min, dirty, 18 °C – 25 °C) to the declared values, and consequently, can be called bactericidal on *Enterococcus hirae* on carriers.
The product **Nocolyse** is capable of reducing the number vegetative yeast cells and the number of mould spores of the relevant organisms on carriers under defined conditions (EN 13697:2015+A1:2019 – concentrated, 15 min and 60 min, dirty, 18 °C – 25 °C) to the declared values, and consequently, can be called yeasticidal and fungicidal on carriers.
The product **Nocolyse** is capable of reducing the number of viable mycobacterial cells of the relevant test organisms under defined conditions (EN 14348:2005 – concentrated, 60 min, clean and dirty conditions, 20 °C) to the declared values, and consequently, can be called mycobactericidal and tuberculocidal.
The product **Nocolyse** is capable of reducing the number of viable bacterial spores of the relevant organism *Clostridium sporogenes* under defined conditions (EN 13704:2018 – concentrated, 60 min, clean and dirty conditions 20 °C) to the declared values, and consequently, can be called sporicidal on *Clostridium sporogenes.*

15.12.2020, Hodonín

Approved by: Mgr. Mirka Horáková, Leader of Study

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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

Copy No.: 1
Issue No.: 1

Test report No.: S59/2022 - 3

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

Nocolyse

Sample ID:	S59/2022	Page.: 1
Sample name:	**Nocolyse**	From pages: 6
Client:	OXY´PHARM, 829 Rue Marcel Paul, 94500 Champigny S/Marne, Francie	
Manufacturer:	OXY´PHARM, 829 Rue Marcel Paul, 94500 Champigny S/Marne, Francie	
Sampling point:	SC REMIX COM SRL, Ing. Teodorescu Street 37, sector 6, 040035 Bucharest, Romania	

Incoming date:
28.2.2022

Delivery date:
27.7.2022

TRADUCĂTOR AUTORIZAT Engleză, Italiană GĂMAN RAMONA-CRISTINA Autorizație MINISTERUL JUSTIȚIEI

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

Sample ID:	S59/2022	Sampling date:	4.1.2022
Sample name:	**Nocolyse**	Sample delivered:	28.2.2022
Sampled:	by client	Testing date:	13.7. - 18.7.2022
Sampling point:	SC REMIX COM SRL	Delivered amount:	2x1 l
Client:	OXY´PHARM	Page:	2

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

Identification of the sample:

Name of the product:	**Nocolyse**
Batch number (Lot):	B040122N/1
Date of manufacture:	01/2022
Expiry date:	01/2024
Manufacturer:	OXY´PHARM, 829 Rue Marcel Paul, 94500 Champigny S/Marne, Francie
Incoming date:	28.2.2022
Storage conditions:	+5 °C - +30 °C
Active ingredients:	CAS:7722-84-1, Hydrogen peroxide, 6%

Experimental conditions: **Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents by suspension method**

SOP:	SOP-M-19-00 (EN 17126:2018)	
Period of analysis:	13. 7. 2022 - 18.7.2022	
Test temperature:	20°C ± 1°C	
Test method:	membrane filtration method	
Filtration diluent:	rinsing liquid	
Appearance of the product:	colourless liquid	
Product diluent:	distilled water	
Test concentration:	97 %	
Contact time:	60 min	
Interfering substances:	0,3 g/l BSA (clean conditions)	
Test organisms:	*Bacillus cereus*	DSM 106266
	Clostridium difficile ribotype 027	DSM 27147
Incubation conditions:	37 °C ± 1 °C, 24-48 hours	
	37 °C ± 1 °C, 5 days	

Test procedure:
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

Note:
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).

The standard:
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*

Sample ID:	S59/2022	Sampling date:	4.1.2022
Sample name:	**Nocolyse**	Sample delivered:	28.2.2022
Sampled:	by client	Testing date:	13.7. - 18.7.2022
Sampling point:	SC REMIX COM SRL	Delivered amount:	2x1 l
Client:	OXY´PHARM	Page:	3

The Number of CFU in the tested product: 0 CFU/ml

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

Test suspensions:

Dilutions	V1	V2	lgN	lgN_0
10^{-6}	>165	>165		
10^{-7}	39	46	8,63	6,63
		$\Phi = 4{,}25 \times 10^{8}$	$7{,}17 \leq lgN \leq 7{,}7$	$5{,}17 \leq lgN_0 \leq 5{,}7$

Verification of methodology

Validation of suspension N_{V0}		Validation of suspenzion N_{Vb}		Membrane filtration validation (B)	
Vc1	115	Vc1	116	Vc1	136
Vc2	125	Vc2	129	Vc2	88
30	$\leq 120 \leq$ *160*	122,5	$\geq$ *0,5 N_{V0}*	112	$\geq$ *0,5 N_v*

Validation of selected experimental conditions (A)

Testing conditions	Vc1	Vc2	ø A	
60 min, 0,3 g/l BSA (clean conditions), 20°C	110	92	101	$\geq$ *0,5 N_{V0}*

Method validation (C)

Testing conditions	Vc1	Vc2	ø C	
97 %, 60 min, 0,3 g/l BSA (clean conditions), 20°C	122	99	110,5	$\geq$ *0,5 N_{V0}*

Testing the efficacy of chemical disinfectant

Testing conditions	**Dilution after test procedure**	**V1**	**V2**	**lgNa** lgNa=lg(ΦAx10)	**lgR** **lgN_0=6,63**
97 %, 60 min, 0,3 g/l BSA (clean conditions), 20°C	**10^0**	**<14**	**<14**	**<2,15**	**>=4,48**

Note: Vc = value is the number of cfu per ml, Φ = average Vc1 a Vc2 (1. + 2. duplicate Vc values), N = the number of cfu/ml of the spore test suspension, N0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0"), Na = the number of surviving spores per ml in the test mixture at the end of the contact time and before the membrane filtration, Nv = the number of cfu/ml of the test suspension for validation, Nv0= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0"), A,B,C = the number of surviving spores per ml in control tests (A – experimental conditions control, B – membrane filtration validation, C – method validation), R = N0 / Na or lg R = lg N0 – lg Na the reduction in viability

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

Sample ID:	S59/2022	Sampling date:	4.1.2022
Sample name:	**Nocolyse**	Sample delivered:	28.2.2022
Sampled:	by client	Testing date:	13.7. - 18.7.2022
Sampling point:	SC REMIX COM SRL	Delivered amount:	2x1 l
Client:	OXY´PHARM	Page:	4

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

Test suspensions:

Dilutions	V1	V2	lgN	lgN_0
10^{-6}	152	146		
10^{-7}	17	16	8,18	6,18
		$\Phi = 1,5x10^8$	$7,17 \leq lgN \leq 7,7$	$5,17 \leq lgN_0 \leq 5,7$

Verification of methodology

Validation of suspension N_{V0}		Validation of suspenzion N_{Vb}		Membrane filtration validation (B)	
Vc1	46	Vc1	55	Vc1	49
Vc2	37	Vc2	28	Vc2	31
30	$\leq 41,5 \leq$ *160*		$41,5 \geq 0,5\ N_{V0}$		$40 \geq 0,5\ N_V$

Validation of selected experimental conditions (A)

Testing conditions	Vc1	Vc2	ø A	
60 min, 0,3 g/l BSA (clean conditions), 20°C	43	35	39	$\geq 0,5\ N_{V0}$

Method validation (C)

Testing conditions	Vc1	Vc2	ø C	
97 %, 60 min, 0,3 g/l BSA (clean conditions), 20°C	51	29	40	$\geq 0,5\ N_{V0}$

Testing the efficacy of chemical disinfectant

Testing conditions	**Dilution after test procedure**	**V1**	**V2**	**lgNa** lgNa=lg(ΦAx10)	**lgR** lgN_0=6,18
97 %, 60 min, 0,3 g/l BSA (clean conditions), 20°C	10^0	**<14**	**<14**	**<2,15**	**>=4,03**

Note: Vc = value is the number of cfu per ml, Φ = average Vc1 a Vc2 (1. + 2. duplicate Vc values), N = the number of cfu/ml of the spore test suspension, N0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0"), Na = the number of surviving spores per ml in the test mixture at the end of the contact time and before the membrane filtration, Nv = the number of cfu/ml of the test suspension for validation, Nv0= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0"), A,B,C = the number of surviving spores per ml in control tests (A – experimental conditions control, B – membrane filtration validation, C – method validation), R = N0 / Na or lg R = lg N0 – lg Na the reduction in viability

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GĂMAN RAMONA-CRISTINA
Autorizație MINISTERUL JUSTIȚIEI

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

Sample ID:	S59/2022	Sampling date:	4.1.2022
Sample name:	**Nocolyse**	Sample delivered:	28.2.2022
Sampled:	by client	Testing date:	13.7. - 18.7.2022
Sampling point:	SC REMIX COM SRL	Delivered amount:	2x1 l
Client:	OXY´PHARM	Page:	5

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

Strain	Test conditions	lgR	lgR
Bacillus cereus DSM 106266	97 %, 60 min, 0,3 g/l BSA (clean conditions), 20°C	≥4	**> 4**
Clostridium difficile ribotype 027 DSM 27147	97 %, 60 min, 0,3 g/l BSA (clean conditions), 20°C	≥4	**> 4**

Note: Vc = value is the number of cfu per ml, Φ = average Vc1 a Vc2 (1. + 2. duplicate Vc values), N = the number of cfu/ml of the spore test suspension, N0 = the number of cfu/ml of the test suspension at the beginning of the contact time (time „0"), Na = the number of surviving spores per ml in the test mixture at the end of the contact time and before the membrane filtration, Nv = the number of cfu/ml of the test suspension for validation, Nv0= the number of cfu/ml of the test suspension in the mixture A,B,C at the beginning of the contact time (time „0"), A,B,C = the number of surviving spores per ml in control tests (A – experimental conditions control, B – membrane filtration validation, C – method validation), R = N0 / Na or lg R = lg N0 – lg Na the reduction in viability

Prepared by: Hana Konevalíková, Lab. Technican

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

Sample ID: S59/2022
Sample name: **Nocolyse**
Sampled: by client
Sampling point: SC REMIX COM SRL
Client: OXY´PHARM

Sampling date: 4.1.2022
Sample delivered: 28.2.2022
Testing date: 13.7. - 18.7.2022
Delivered amount: 2x1 l
Page: 6

Conslusion:

The tested product: **Nocolyse**
Batch number: B040122N/1
Standard: EN 17126:2018
Test method: membrane filtration method

For conditions: 97 %, 60 min, 0,3 g/l BSA (clean conditions), 20°C
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 consenquently, can be called sporicidal on *Bacillus cereus* and *Clostridium difficile*.

Approved by: Ing. Barbora Stoklásková, Leader of Study

Hodonín, 27.7.2022

Ing. Jana Šlitrová, Head of Laboratory

Traducere din limba engleză

Chemila
Chemila, spol s.r.o. Za Drahou 4386/3, Hodonin 69501, Telefon +420518340919, chemila@chemila.cz
Laborator de chimie și microbiologie, Laborator de testare nr. 1273 certificat de către Institutul de Acreditare Ceh conform CSN EN ISO/IEC 17025:2018.

Exemplar nr. 1
Ediție nr. 1

Raport de Testare Nr. S59/2022 - 3

STABILIREA ACTIVITĂȚII SPORICIDE (EN 17126:2018)
A PRODUSULUI **NOCOLYSE**

Nr. Identificare probă:	S59/2022
Denumire probă:	**Nocolyse**
Client:	OXY'PHARM SARL, 829 Rue Marcel Paul, 94500 Champigny S/Mame, Franța
Producător:	OXY'PHARM, 829 Rue Marcel Paul, 94500 Champigny S/Mame, Franța
Punct de prelevare probe:	SC REMIX COM SRL, strada Ing. Teodorescu nr. 37, sector 6, București, România

Data intrării: 28.2.2022 Data livrării: 27.7.2022

Hodonin, 15.3.2022

Ștampilă Laborator de testare certificat de către Institutul de Acreditare Ceh
Chemila, spol s.r.o.
Za Drahou 4386/3, Hodonin 69501

Ing. Jana Slitrova, Șef laborator
Semnătură indescifrabilă

Rezultatele testelor se referă numai la probele menționate în raportul de testare. Raportul de testare poate fi reprodus doar în întregime, pe bucăți numai cu permisiunea scrisă a laboratorului. În cazul în care laboratorul nu este responsabil pentru eșantionare, rezultatele se referă la probe așa cum au

fost primite. Laboratorul nu acordă nicio garanție pentru identitatea probelor neprelevate de personalul de laborator. Clientul este responsabil pentru informațiile furnizate despre probe.

Descriere: *Testarea eficacității dezinfectanților chimici și antisepticelor*

Nr. identificare probă: S59/2022	Dată prelevare: 4.1.2022
Denumire probă: **Nocolyse**	Probă livrată: 28.2.2022
Prelevat: de către client	Dată testare: 13.7 – 18.7.2022
Punct de prelevare probe: SC REMIX COM SRL	Cantitate livrată: 2 x 1 1
Client: OXY'PHARM	Pagina:2

Obiectul testării:
Stabilirea activității sporicide a produsului.

Identificarea probei:

Denumirea produsului:	**Nocolyse**
Numărul lotului:	B040122N/1
Data producției:	01/2022
Data expirării:	01/2024
Producător:	OXY'PHARM SARL, 829 Rue Marcel Paul, 94500 Champigny S/Mame, Franța
Data intrării:	28.2.2022
Condiții de depozitare:	+5 °C - +30 °C
Ingrediente active:	CAS 7722-84-1 peroxid de hidrogen 6%

Condiții experimentale:	**Testarea eficienței de dezinfectare a agenților chimici dezinfectanți prin metoda de suspensie** SOP-M-19-00 (EN 17126:2018)
Perioada analizei:	13.7.2022 – 18.7.2022
Temperatura de testare:	20°C± 1 °C
Metoda de testare:	metoda filtrării membranare
Diluant de filtrare:	lichid de clătire
Aspectul produsului:	lichid incolor
Diluant produs:	apă distilată
Concentrație de testare:	97 %
Perioada de contact:	60 min
Substanțe de interferență:	BSA (albumină serică bovină) 3,0 g/l (condiții de curățenie)
Organisme de testare:	*Bacillus cereus* DSM 106266

Clostridium difficile ribotype 027 DSM 27147

Condiții de incubare: 37 °C ± 1 °C, 24-48 ore
37 °C ± 1 °C, 5 zile

Procedura de testare:

1. Prepararea suspensiei de testare
2. Prepararea soluțiilor de testare a produsului
3. Testarea suspensiei cantitative
4. Incubare și calcul
5. Exprimarea și interpretarea rezultatelor

Notă:
Activitate sporicidă - capacitatea unui produs de a realiza o reducere a numărului de spori bacterieni aparținând tulpinilor de referință *Bacillus subtilis* și *Bacillus cereus* în condiții definite cu o reducere de cel puțin 4 lg (10^4).
Activitate sporicidă împotriva *Clostridium difficile* - capacitatea unui produs de a realiza o reducere a numărului de spori bacterieni aparținând tulpinii de referință *Clostridium difficile* în condiții definite cu o reducere de cel puțin 4 lg (10^4).

Standard:
EN 17126:2018 Dezinfectanți chimici și antiseptice – Testarea suspensiei cantitative pentru evaluarea activității sporicide a dezinfectanților chimici în domeniul medical - Metodă de testare și cerințe (etapa 2, pasul 1), decembrie 2018

Numărul de CFU din produsul de testare: 0 CFU/ml

Testarea eficacității dezinfectantului chimic **Nocolyse** pe *Bacillus cereus* DSM 106266

Suspensiile de testare:

Diluții	V1	V2	lgN	lgN_0
10^{-6}	>165	>165		
10^{-7}	39	46	8,63	6,63
$\Phi = 4{,}25 \times 10^8$			$7{,}17 \leq lgN \leq 7{,}7$	$5{,}17 \leq lgN_0 \leq 5{,}7$

Verificarea metodologiei

Validarea suspensiei N_{v0}		Validarea suspensiei N_{vb}		Validare filtrare membranară (B)	
Vc1	115	Vc1	116	Vc1	136
Vc2	125	Vc2	129	Vc2	88
$30 \leq 120 \leq 160$		$122{,}5 \geq 0{,}5\ N_{v0}$		$112 \geq 0{,}5\ N_{v0}$	

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Validarea condițiilor experimentale selectate (A)

Condiții de testare	Vc1	Vc2	Ø A
60 min, 0,3 g/l BSA (condiții de curățenie), 20 ^{0}C	110	92	101 $\geq 0,5\ N_{v0}$

Validarea metodei (C)

Condiții de testare	Vc1	Vc2	Ø C
97 %, 60 min, 0,3 g/l BSA (condiții de curățenie), 20 ^{0}C	122	99	110,5 $\geq 0,5\ N_{v0}$

Testarea eficacității dezinfectantului chimic

Condiții de testare	**Diluție după procedura de testare**	**Vc1**	**Vc2**	**lgNa lgNa=lg(Φax10)**	**lgR lgN$_0$=6,63**
97 %, 60 min, 0,3 g/l BSA (condiții de curățenie), 20 ^{0}C	**10^0**	**<14**	**<14**	**<2,15**	**>=4,48**

Notă: Vc = valoarea este numărul cfu per ml, Φ = media Vcl a Vc2 (1. + 2. valori Vc duplicate), N = numărul cfu/ml al suspensiei de testare a sporilor, N0 = numărul cfu/ml al suspensiei de testare la începutul perioadei de contact (timpul „0“), Na = numărul de spori rămași per ml în amestecul de testare la sfârșitul perioadei de contact și înainte de filtrarea membranară, Nv = numărul cfu/ml al suspensiei de testare pentru validare, Nv0= numărul cfu/ml al suspensiei de testare în amestecul A,B,C la începutul perioadei de contact (timpul „0“), A,B ,C = numărul de spori rămași per ml în testele de control (A - controlul condițiilor experimentale, B - validarea filtrării membranare, C - validarea metodei), R = N0 / Na sau Ig R = Ig N0 - lg Na reducerea viabilității

CFU = unități formatoare de colonii

Testarea eficacității dezinfectantului chimic **Nocolyse** pe *Clostridium difficile ribotype 027* DSM 27147

Suspensiile de testare:

Diluții	V1	V2	lgN	lgN$_0$
10^{-6}	152	146		
10^{-7}	17	16	8,18	6,18
		$\Phi = 1,5 \times 10^8$	$7,17 \leq lgN \leq 7,7$	$5,17 \leq lgN_0 \leq 5,7$

Verificarea metodologiei

Validarea suspensiei N_{v0}	Validarea suspensiei N_{vb}	Validare filtrare membranară (B)

Vc1	46	Vc1	55	Vc1	49
Vc2	37	Vc2	28	Vc2	31
30 ≤ 41,5 ≤ *160*		41,5 ≥ *0,5* N_{v0}		40 ≥ *0,5* N_{v0}	

Validarea condițiilor experimentale selectate (A)

Condiții de testare	Vc1	Vc2	Ø A
60 min, 0,3 g/l BSA (condiții de curățenie), 20 ^{0}C	43	35	39 ≥ *0,5* N_{v0}

Validarea metodei (C)

Condiții de testare	Vc1	Vc2	Ø C
97 %, 60 min, 0,3 g/l BSA (condiții de curățenie), 20 ^{0}C	51	29	40 ≥ *0,5* N_{v0}

Testarea eficacității dezinfectantului chimic

Condiții de testare	**Diluție după procedura de testare**	**Vc1**	**Vc2**	**lgNa lgNa=lg(Φax10)**	**lgR lgN$_0$=6,18**
97 %, 60 min, 0,3 g/l BSA (condiții de curățenie), 20 ^{0}C	**10^0**	**<14**	**<14**	**<2,15**	**>=4,03**

Notă: Vc = valoarea este numărul cfu per ml, Φ = media Vcl a Vc2 (1. + 2. valori Vc duplicate), N = numărul cfu/ml al suspensiei de testare a sporilor, N0 = numărul de cfu/ml al suspensiei de testare la începutul perioadei de contact (timpul „0“), Na = numărul de spori rămași per ml în amestecul de testare la sfârșitul perioadei de contact și înainte de filtrarea membranară, Nv = numărul de cfu/ml al suspensiei de testare pentru validare, Nv0= numărul de cfu/ml al suspensiei de testare în amestecul A,B,C la începutul perioadei de contact (timpul „0“), A,B ,C = numărul de spori rămași per ml în testele de control (A - controlul condițiilor experimentale, B - validarea filtrării membranare, C - validarea metodei), R = N0 / Na sau Ig R = Ig N0 - lg Na reducerea viabilității

Evaluarea activității SPORICIDE (EN 17126:2018) a produsului **Nocolyse**

Tulpină	Condiții de testare	lgR	lgR
Bacillus cereus DSM 106266	97 %, 60 min, 0,3 g/l BSA (condiții de curățenie), 20 ^{0}C	≥4	>4
Clostridium difficile ribotype 027 DSM 27147	97 %, 60 min, 0,3 g/l BSA (condiții de curățenie), 20 ^{0}C	≥4	>4

Notă: Vc = valoarea este numărul cfu per ml, Φ = media Vcl a Vc2 (1. + 2. valori Vc duplicate), N = numărul cfu/ml al suspensiei de testare a sporilor, N0 = numărul cfu/ml al suspensiei de testare la începutul perioadei de contact (timpul „0“), Na = numărul de spori rămași per ml în amestecul de testare la sfârșitul perioadei de contact și înainte de filtrarea membranară, Nv = numărul cfu/ml al suspensiei de testare pentru validare, Nv0= numărul cfu/ml al suspensiei de testare în amestecul

A,B,C la începutul perioadei de contact (timpul „0“), A,B ,C = numărul de spori rămași per ml în testele de control (A - controlul condițiilor experimentale, B - validarea filtrării membranare, C - validarea metodei), R = N0 / Na sau Ig R = Ig N0 - lg Na reducerea viabilității

Pregătit de către: Hana Konevalikov, Tehnician laborator

Concluzie:
Produsul testat: **Nocolyse**
Număr lot: B040122N/1
Standard: EN 17126:2018
Metoda de testare: metoda de filtrare membranară
Pentru condiții: 97 %, 60 min, 0,3 g/1 BSA (condiții de curățenie), 20°C
Bacillus cereus, Clostridium difficile ribotype 027
eficacitatea este confirmată.
Produsul testat este capabil să reducă numărul de celule viabile ale organismelor relevante în condiții definite la valorile declarate și, în consecință, poate fi numit sporicid pe *Bacillus cereus* și *Clostridium difficile*.

Aprobat de: Ing. Barbora Stoklaskova, Coordonator studiu

27.7.2022, Hodonin

Ștampilă Laborator de testare certificat de către
Institutul de Acreditare Ceh
Chemila, spol s.r.o.
Za Drahou 4386/3, Hodonin 69501
Semnătură indescifrabilă

*Subsemnata **GĂMAN RAMONA CRISTINA** interpret si traducător autorizat pentru limbile străine engleză şi italiană, în temeiul autorizaţiei nr. **7332** din **2002** eliberată de Ministerul Justiţiei din România, certific exactitatea traducerii efectuate din limba engleză în limba română.*

Traducător,
Ramona-Cristina Găman

Toulouse, June 4th 2021

ASSAY REPORT N° 21-1716

STUDY 20-2794

STANDARD NF EN 17272 (Avril 2020)
Chemical disinfectants and antiseptics –
Methods of airborne room disinfection by automated process – Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities

Medical area
Clean conditions

Client	**OXY'PHARM** **829 rue Marcel Paul** **94500 CHAMPIGNY SUR MARNE** **FRANCE**
Assay laboratory	**FONDEREPHAR** **Faculté des Sciences Pharmaceutiques** **35 Chemin des Maraîchers** **31062 TOULOUSE cedex 9** **FRANCE**

Pr **Christine ROQUES** Study Manager	Dr **Jocelyne BACARIA** Quality Manager
PO Catherine FEUILLOLAY Signature numérique de Catherine FEUILLOLAY Date : 2021.06.10 12:06:04 +02'00'	Jocelyne BACARIA Signature numérique de Jocelyne BACARIA Date : 2021.06.10 12:34:47 +02'00'

1. Test Laboratory

Fondation pour le Développement de la recherche en Pharmacie (FONDEREPHAR)
Faculté des Sciences Pharmaceutiques, 35 chemin des Maraîchers 31062 Toulouse cedex 9, France

2. Identification of the aerial disinfection system

Device : **NOCOSPRAY 2**
Serial number :172X731

Disinfectant : **NOCOLYSE NEUTRAL 6%®**
Batch : A281020N/1
Exp.: 10/2022
Receipt : Nov/03/2020

Disinfectant : **NOCOLYSE NEUTRAL 6%®**
Batch : A050121N/2
Exp. : 01/2023
Receipt: Jan/08/2021

Concentration of product: 5mL/m^3
One treatment - Waiting time 120 minutes after the end of diffusion
Amount of disinfectant diffusion ≈ 162,5 mL
Time of diffusion : 9 minutes 45 secondes

Promotor : OXY'PHARM

Storage conditions: Ambiant temperature
Period of testing: November 2020 – May 2021

Actives Substances: Hydrogen peroxide (6%)

3. Experimental Conditions

a. Tests micro-organisms

- Bactericidal activity :
  - *Acinetobacter baumanii* CIP 7034
  - *Staphylococcus aureus* CIP 4.83
  - *Enterococcus hirae* CIP 58.55
  - *Escherichia coli* CIP 54.127

- Fungicidal activity :
  - *Candida albicans* DSM 1386
  - *Aspergillus brasiliensis* CBS 733.88

- Sporicidal activity :
  - *Bacillus subtilis* CIP 52.62
  - *Clostridium difficile* NCTCC 13366

- Mycobactericidal activity :
  - *Mycobacterium terrae* ATCC 15755
  - *Mycobacterium avium* ATCC 15769

- Virucidal activity (virus/receiving cells):

Adenovirus/HELA Cells

Virus
Origin: ATCC
ATCC reference: VR-5
Batch number supplier: 58486654
Internal number Batch: SS-7-180611 (passage N°7)

Receiving cells
Origin: ATCC
ATCC reference: CCL-2
Batch number ATCC: 4440136
Internal number Batch: WCB-140613 (passage N°30)

Murine Norovirus souche S99/RAW264.7 cells:

Virus
Origin : Friedrich Loefler Institut Berlin
Supplier reference: RVB-651
Batch number supplier: 4/200409/220409
Internal number Batch: SS-5-110419 (passage N°5)

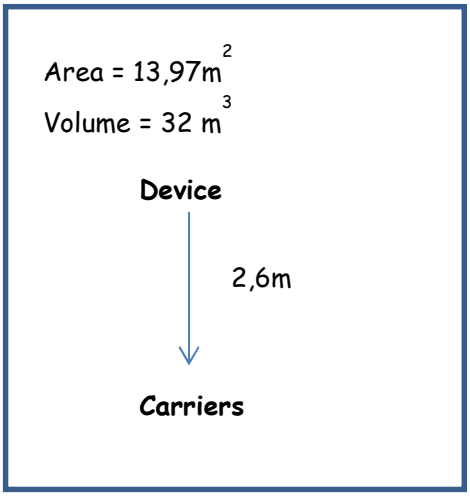
Receiving cells
Origin : ATCC
Designation : RAW264.7
ATCC reference: TIB-71
Batch number ATCC: 5822175
Internal number Batch: WCB-210912 (passage N°35)

b. Carriers

The selected tests surfaces are stainless steel discs, flats, corresponding to the requirements of paragraph 5.2.3.2 of the standard. The supplier is MERCIER CLAUSSE (France).

c. Conditions of aerial disinfection system use

- Room :



Relative humidity ranging from 50% to 64% (see results).
Initial temperatures ranging from 19,3°C to 20,5°C (see results).

Test room volume : 32m³.

Distance between the appartus and the carriers : 2,6m (tableau B.1), 1,15m from floor.

d. Diluants, culture media and membranes

Interfering substances
1/20 reconstituted milk (Internal preparation - Batches 9780 Exp. Nov/14/2020, 9883 Exp. Dec/16/2020, 10001 Exp. Jan/15/21 and 10155 Exp. Mar/15/21)
BSA fraction V 0,3g/l (Internal preparation - Batches 351, 361, 362, 367, 368, 383, 390)

Diluants
Suspension preparation : Tryptone salt (Biomérieux - Batches 1843680 Exp. Jun/22/2021 and 1856370, Exp. Jul/16/2021) or Water for Injectable Preparations (WIP)* (interference of product with Tryptone-salt) (Cooper - Batch 19MKA300 Exp. Sept/2021)
Diluant for *A. brasiliensis* (Internal preparation - Batch 52 Exp. Mar/07/21)
Recovery solution + 0,5% Tween80 (Internal preparation - Batches 9851, 9869, 9880, 10008, 10029, 10075, 10096, 10097)
Recovery solution (viruses): EMEM (Batches N°2848, N°2849, N°2856 and 2857)

Filtration membranes
Nitrocellulose membranes 0,45 µm (Millipore – white / Batches F0JB71371C and F0MB14755C – black / Batch F9HA42174)

Culture media
Malt Extract agar (Internal preparation - Batches 10015 Exp. Jan/29/21, 10030 Exp. Feb/04/21 and 10156 Exp. Mar/15/21)
Trypcase soy agar (Biomérieux - Lot 1008219370 Exp. Jan/30/2022 and 1008383190 Exp.

May/05/2022)
Middlebrook agar + OADC (Internal preparation - Batches 9775 Exp. Nov/14/2020, 9850 Exp. Dec/04/2020 and 9875 Exp. Dec/12/2020)
BHIYT-L Agar (Counting of *Clostridium difficile*) (Internal preparation – Batch 10080 Exp. Feb/18/2021)
EMEM (Batches N°2848, N°2849, N°2856 and 2857)

e. Virucidal activity: validation and titration

Control of sensitivity of cells to virus

- Add one volume of solution S or PBS + one volume of cellular suspension at 2.10^5 cells/ml for one hour in water bath at 36°C±1°C
- The cells are centrifuged at 1600trs/min for 10 min and resuspended in culture media
- The virus is diluted from 1/10 to 1/10 on a 96-well microplate (10 dilutions)
- Add 100 µl of cell suspension treated (Solution S) or not treated (PBS control) to each well of the microplate
- Incubate for 72 hours

The difference of title reduction between cells treated by the solution S and cells treated by PBS shall be < 1 lg.

Control of efficiency for suppression of disinfectant activity

- Add 1 volume of BSA + 1 volume of virus suspension + 1 volume of solution S or distilled water
- Leave the mixture in the ice bath for 60 min at room temperature

Titration method

- Titrate the virus (method titration on cell in suspension) by following steps:
- Serial dilutions (1/10) are realized with culture medium in the glass tube
- Transfer 0,1 ml of each dilution into eight wells of a microplate plaque
- The last row of eight wells will receive 0,1 ml of culture medium (control untreated cells)
- Add 0,1 ml of cell suspension at 2.10^5cell/ml.
- Incubate for 72 hours at 36 ° C ± 1 ° C under 5% CO_2 ± 2%.
- The viral cytopathic effect is read by using an inverted microscope

The estimated of infectious unite is determined by method KARBER-SPAERMAN calculating the negative logarithm of 50% endpoint (lgDIC50) by the following formula:

lgDICT50 = negative logarithm of the highest concentration of virus - [(Sum of% affected to each dilution/100 - 0.5) X (lg dilution)]

4. Assays

a. Bactericidal activity

- **5 mL / m^3 – waiting 120 minutes – Batch A050121N/2**

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 UFC/ spot 50µL (dilution/filtration – disc in agar)	Log reduction - Mean
		n1/N1	n2/N2	n3/N1			
	5.10^7 – 2.10^9	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10^6		
S. aureus* **Assay Feb/17/2021** **20,2°C / HR 53%**	$4,90.10^8$	d1 : 56/49 d2 : 53/49	d1 : 61/54 d2 : 54/54	d1 : 58/49 d2 : 46/49	d1 : $7,85.10^6$ d2 : $9,25.10^6$ **T** = $8,55.10^6$	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,93 R2 : 6,93 R3 : 6,93 **R = 6,93**
A. baumanii* **Assay Feb/17/2021** **20,2°C / HR 53%**	$5,80.10^8$	d1 : 54/58 d2 : 52/58	d1 : 51/61 d2 : 55/61	d1 : 70/58 d2 : 51/58	d1 : $6,90.10^6$ d2 : $5,70.10^6$ **T** = $6,30.10^6$	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,80 R2 : 6,80 R3 : 6,80 **R = 6,80**

T: counting of micro-organisms on the discs.
N_1 : counting of test suspension by pour plate technique - N_2 : counting of test suspension by filtration method
n_1 : counting to search inhibitor effect in agar medium - n_2 : counting to search inhibitor effect on membrane filtration - n_3 : counting to search inhibitor effect after inclusion of disc in agar medium
n'_1 : number of survival micro-organisms in 100mL of tryptone-salt - n'_2 : number of micro-organisms after inclusion of the disc in agar medium.
$n'_1 + n'_2$: total number of survival micro-organisms on the carrier surface. d1 : disc N°1 / d2 : disc N°2 / d3 : disc N°3

- **5 mL / m³ – waiting 120 minutes – Batch A281020N/1**

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 UFC/ spot 50µL (dilution/filtration – disc in agar)	Log reduction - Mean
		n1/N1	n2/N2	n3/N1			
	5.10^7 – 2.10^9	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10^6		
E. coli **Assay Nov/17/2020** **19,5°C / RH 58%**	$2,63.10^9$	d1 : 29/32 d2 : 28/32	d1 : 31/45 d2 : 34/45	d1 : 33/32 d2 : 30/32	d1 : $8,35.10^6$ d2 : $9,60.10^6$ **T** = $8,98.10^6$	d1 : 0 + 0 d2 : 1 + 0 d3 : 0 + 0	R1 : 6,95 R2 : 6,95 R3 : 6,95 **R = 6,95**
E. hirae **Assay Nov/17/2020** **19,5°C / RH 58%**	$3,17.10^8$	d1 : 75/34 d2 : 70/34	d1 : 79/39 d2 : 108/39	d1 : 95/34 d2 : 98/34	d1 : $1,19.10^7$ d2 : $0,93.10^7$ **T** = $1,06.10^7$	d1 : 0 + 0 d2 : 810 + 0 d3 : 470 + 0	R1 : 7,03 R2 : 4,12 R3 : 4,35 **R = 5,17**

T: counting of micro-organisms on the discs.
N_1 : counting of test suspension by pour plate technique - N_2 : counting of test suspension by filtration method
n_1 : counting to search inhibitor effect in agar medium - n_2 : counting to search inhibitor effect on membrane filtration - n_3 : counting to search inhibitor effect after inclusion of disc in agar medium
n'_1 : number of survival micro-organisms in 100mL of tryptone-salt - n'_2 : number of micro-organisms after inclusion of the disc in agar medium.
$n'_1 + n'_2$: total number of survival micro-organisms on the carrier surface.
d1 : disc N°1 / d2 : disc N°2 / d3 : disc N°3

b. Fungicidal activity

- **Treatment 5 mL / m^3 – waiting 120 minutes – Batch A281020N/1**

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 UFC/ spot 50µL (dilution/filtration – disc in agar)	Log reduction - Mean
		n1/N1	n2/N2	n3/N1			
	2.10^7 – 1.10^8	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10^5		
C. albicans **Assay Jan/06/21** **19,5°C / RH 50%**	$6,40.10^7$	d1 : 57/64 d2 : 62/64	d1 : 62/66 d2 : 53/66	d1 : 61/64 d2 : 52/64	d1 : $8,65.10^5$ d2 : $8,60.10^5$ **T** = $8,63.10^5$	d1 : 30 + 0 d2 : 33 + 0 d3 : 7 + 0	R1 : 4,46 R2 : 4,42 R3 : 5,09 **R = 4,66**

T: counting of micro-organisms on the discs.
N_1 : counting of test suspension by pour plate technique - N_2 : counting of test suspension by filtration method
n_1 : counting to search inhibitor effect in agar medium - n_2 : counting to search inhibitor effect on membrane filtration - n_3 : counting to search inhibitor effect after inclusion of disc in agar medium
n'_1 : number of survival micro-organisms in 100mL of tryptone-salt - n'_2 : number of micro-organisms after inclusion of the disc in agar medium.
$n'_1 + n'_2$: total number of survival micro-organisms on the carrier surface.
d1 : disc N°1 / d2 : disc N°2 / d3 : disc N°3

- **Treatment 5 mL / m^3 – waiting 120 minutes – Batch A050121N/2**

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 UFC/ spot 50µL (dilution/filtration – disc in agar)	Log reduction - Mean
		n1/N1	n2/N2	n3/N1			
	5.10^6 – 1.10^7	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10^5		
A. brasiliensis **Assay Feb/17/2021** **20,2°C / RH 53%**	$1{,}12.10^7$	d1 : 50/45 d2 : 51/45	d1 : 29/30 d2 : 23/30	d1 : 29/45 d2 : 38/45	d1 : $1{,}04.10^6$ d2 : $1{,}03.10^6$ **T** = $1{,}04.10^6$	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,02 R2 : 6,02 R3 : 6,02 **R = 6,02**

T: counting of micro-organisms on the discs.
N_1 : counting of test suspension by pour plate technique - N_2 : counting of test suspension by filtration method
n_1 : counting to search inhibitor effect in agar medium - n_2 : counting to search inhibitor effect on membrane filtration - n_3 : counting to search inhibitor effect after inclusion of disc in agar medium
n'_1 : number of survival micro-organisms in 100mL of tryptone-salt - n'_2 : number of micro-organisms after inclusion of the disc in agar medium.
$n'_1 + n'_2$: total number of survival micro-organisms on the carrier surface.
d1 : disc N°1 / d2 : disc N°2 / d3 : disc N°3

c. Sporicidal activity

- **Treatment 5 mL / m^3 – waiting 120 minutes – Batch A050121N/2**

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 UFC/ spot 50µL (dilution/filtration – disc in agar)	Log reduction - Mean
		n1/N1	n2/N2	n3/N1			
	2.10^6 – 5.10^6	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10^5		
B. subtilis* **Assay Jan/26/21** **19,9°C/RH 57%**	$4,30.10^6$	d1 : 40/43 d2 : 38/43	d1 : 37/28 d2 : 35/28	d1 : 50/43 d2 : 37/43	d1 : $1,23.10^5$ d2 : $1,37.10^5$ **T** = $1,30.10^5$	d1 : 11 + 0 d2 : 8 + 0 d3 : 7 + 0	R1 : 4,07 R2 : 4,21 R3 : 4,27 **R = 4,18**
C. difficile* **Assay Jan/21/21** **19,3°C/RH 60%**	$2,05.10^6$	d1 : 15/23 d2 : 19/23	d1 : 18/18 d2 : 17/18	d1 : 13/23 d2 : 13/23	d1 : $0,61.10^5$ d2 : $0,58.10^5$ **T** = $0,60.10^5$	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 1	R1 : 4,78 R2 : 4,78 R3 : 4,78 **R = 4,78**

T: counting of micro-organisms on the discs.
N_1 : counting of test suspension by pour plate technique - N_2 : counting of test suspension by filtration method
n_1 : counting to search inhibitor effect in agar medium - n_2 : counting to search inhibitor effect on membrane filtration - n_3 : counting to search inhibitor effect after inclusion of disc in agar medium
n'_1 : number of survival micro-organisms in 100mL of tryptone-salt - n'_2 : number of micro-organisms after inclusion of the disc in agar medium.
$n'_1 + n'_2$: total number of survival micro-organisms on the carrier surface.
d1 : disc N°1 / d2 : disc N°2 / d3 : disc N°3

d. Mycobactericidal activity

- **Treatment 5 mL / m^3 – waiting 120 minutes – Batch A281020N/1**

Tests microorganisms	N Test suspension (CFU/mL)	Preliminary tests			T Control (CFU/spot - 50µL)	n'1 + n'2 UFC/ spot 50µL (dilution/filtration – disc in agar)	Log reduction - Mean
		n1/N1	n2/N2	n3/N1			
	1.10^7 – 1.10^8	n1 > 0.5 N1	n2 > 0.5 N2	n3 > 0.5 N1	≈ 10^5		
M. terrae **Assay Nov/09/20 18,8°C/RH 51%**	$3,10.10^7$	d1 : 73/76 d2 : 76/76	d1 : 57/67 d2 : 58/67	d1 : 54/76 d2 : 54/76	d1 : $3,23.10^6$ d2 : $3,33.10^6$ **T** = $3,28.10^6$	d1 : 0 + 0 d2 : 0 + 0 d3 : 1 + 0	R1 : 6,52 R2 : 6,52 R3 : 6,52 **R = 6,52**
M. avium **Assay Nov/16/20 18,8°C/RH 63%**	$3,92.10^7$	d1 : 85/88 d2 : 57/88	d1 : 65/61 d2 : 63/61	d1 : 77/88 d2 : 69/88	d1 : $2,66.10^6$ d2 : $2,35.10^6$ **T** = $2,51.10^6$	d1 : 0 + 0 d2 : 0 + 0 d3 : 0 + 0	R1 : 6,40 R2 : 6,40 R3 : 6,40 **R = 6,40**

T: counting of micro-organisms on the discs.
N_1 : counting of test suspension by pour plate technique - N_2 : counting of test suspension by filtration method
n_1 : counting to search inhibitor effect in agar medium - n_2 : counting to search inhibitor effect on membrane filtration - n_3 : counting to search inhibitor effect after inclusion of disc in agar medium
n'_1 : number of survival micro-organisms in 100mL of tryptone-salt - n'_2 : number of micro-organisms after inclusion of the disc in agar medium.
$n'_1 + n'_2$: total number of survival micro-organisms on the carrier surface.
d1 : disc N°1 / d2 : disc N°2 / d3 : disc N°3

e. Virucidal activity

- **Treatment 5 mL / m^3 – waiting 120 minutes – Batch A050121N/2**

- **Adenovirus type 5**

No cytotoxicity was observed on the carrier without treatment which has been pretreated with the aerial disinfection.

Assay May/11/2021 **20,5°C/RH 64%**	Degree of cytopathogenic effect (lgDICT50)	Logarithmic reduction
Sensitivity of cells to virus		
- With treatment (S1)		
Carrier 1	7.88	
Carrier 2	7.63	
Average	7.76	Difference <1 lg.
- Without traitement (S2)		
Carrier 1	7.75	
Efficiency for suppression of disinfectant activity		
- With treatment (D1)		
Carrier1	7.63	
Carrier 2	7.75	Difference <0,5 lg.
Average	7.69	
- Without traitement (D2)		
Carrier 1	7.50	
Test control		
Carrier1	6.25	
Carrier 2	6.88	
Average	6.57	
Assay		
Support 1	1.50	
Support 2	1.50	**5.07**
Support 3	1.50	
Average	1.50	

- Murine Norovirus

No cytotoxicity was observed on the carrier without treatment which has been pretreated with the aerial disinfection.

Assay April/29/2021 **19,4°C/RH 64%**	Degree of cytopathogenic effect (lgDICT50)	Logarithmic reduction
Sensitivity of cells to virus		
- With treatment (S1)		
Carrier 1	6.88	
Carrier 2	6.88	
Average	6.88	Difference <1 lg.
- Without traitement (S2)		
Carrier 1	7.00	
Efficiency for suppression of disinfectant activity		
- With treatment (D1)		
Carrier1	6.50	
Carrier 2	6.63	Difference <0,5 lg.
Average	6.57	
- Without traitement (D2)		
Carrier 1	6.38	
Test control		
Carrier1	6.13	
Carrier 2	6.38	
Average	6.26	
Assay		
Support 1	1.50	
Support 2	2.00	**4.55**
Support 3	1.63	
Average	1.71	

5. Conclusion

According to the conditions of standard NF EN 17272 (April 2020), the couple device/product: NOCOSPRAY 2 serial number 172X731 / NOCOLYSE NEUTRALL 6%® (Batches A281020N/1 Exp. Oct/2022 and A050121N/2 Exp. Jan/2023), for a use in clean conditions, in medical area, led to:

- **A bactericidal activity (log reduction ≥ 5) after a 5 mL/m^3 treatment and 120 minutes of wait on the following strain:**
  - *A. baumanii* CIP 7034
  - *E. coli* CIP 54.127
  - *E. hirae* CIP 58.55
  - *S. aureus* CIP 4.83

- **A fungicidal activity (log reduction ≥ 4) after a 5 mL/m^3 treatment and 120 minutes of wait on the following strain:**
  - *C. albicans* DSM 1386
  - *A. brasiliensis* CBS 733.88

- **A sporicidal activity (log reduction ≥ 4) after a 5 mL/m^3 treatment and 120 minutes of wait on the following strain:**
  - *Bacillus subtilis* CIP 52.62
  - *C. difficile* NCTCC 13366

- **A mycobactericidal activity (log reduction ≥ 4) after a 5 mL/m^3 treatment and 120 minutes of wait on the following strain:**
  - *M. terrae* ATCC 15755
  - *M. avium* ATCC 15769

- **A virucidal activity (log reduction ≥ 4) after a 5 mL/m^3 treatment and 120 minutes of wait on the following strain:**
  - *Adenovirus* type 5 ATCC VR-5
  - *Norovirus Murin* souche S99

The results hold only for the device/product under assay and apply to the sample as received.

FONdation pour le DEveloppement de la REcherche PHARmaceutique

Toulouse, November 26th 2020

STUDY 20 - 2794

TEST REPORT N° 20-1589

Standard NF EN 17272 (April 2020)
Antiseptics and chemical disinfectants - Methods of airborne room disinfection
By automated process
Determination of Virucidal Activity - Human Coronavirus 229E
Medical area
Clean condition

Promotor	**OXY'PHARM** **829 rue Marcel Paul** **94500 CHAMPIGNY SUR MARNE**
Test Laboratory	**FONDEREPHAR** **Faculté des Sciences Pharmaceutiques** **35 Chemin des Maraîchers** **31062 TOULOUSE cedex 9**

Dr. Laila HADDIOUI *Assay Manager* Laila HADDIOUI Signature numérique de Laila HADDIOUI Date : 2022.02.02 09:49:27 +01'00'	Dr **Jocelyne BACARIA** Quality Manager Jocelyne BACARIA Signature numérique de Jocelyne BACARIA Date : 2022.02.02 09:54:16 +01'00'

1. Test Laboratory

Fondation pour le Développement de la recherche en Pharmacie (FONDEREPHAR)
Faculté des Sciences Pharmaceutiques, 35 chemin des Maraîchers 31062 Toulouse cedex 9, France

2. Identification of the aerial disinfection system

Product :	**NOCOLYSE® Neutral 6%**
Batch :	A281020N/1
Expiry date :	10/2022
Date of receipt :	November/03/2020
Internal code :	20-2794-1
Active Substance:	Hydrogen peroxide (6%)
Device :	**NOCOSPRAY**
Serial number :	172X731

Concentration of disinfectant in the room: 5 mL/m^3
One treatment - recovery of the discs after 2 hours waiting at the end of the diffusion.

Promotor :	OXY'PHARM
Storage conditions:	Ambiant temperature
Period of testing:	November 2020

3- Experimental conditions

3-1 Virus/Receiving cells

Virus

Name	Human Coronavirus 229E
Origin :	ATCC
ATCC reference:	VR-740
Batch number supplier:	58505270
Internal number Batch:	SS-2-280520 (passage N°2)

Receiving cells

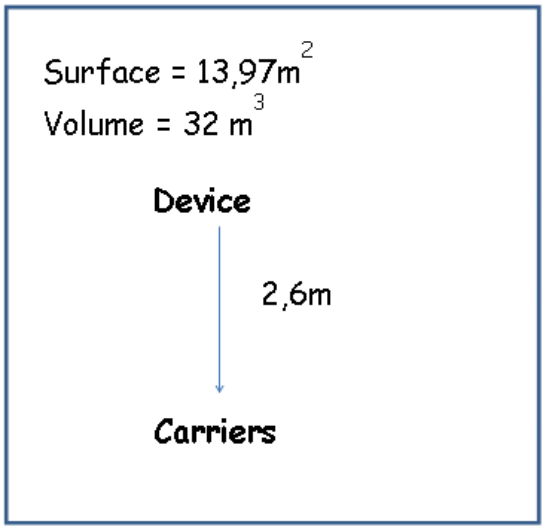
Name	Vero cells
Origin :	ATCC
ATCC reference:	CCL-81
Batch number ATCC:	3372621
Internal number Batch:	WCB-141215 (passage N°30)

3-2 Carriers

The selected tests surfaces are stainless steel discs, flats, corresponding to the requirements of paragraph 5.2.3.1 of the standard. The suppliers are MERCIER CLAUSSE.

3-3 Conditions of use of the device/product

- **Room** :



Relative humidit: start of test 55% - end of test 59% (requirements 40 - 80%).
Temperature: start of test 18.2°C - end of test 19.1°C (requirements 18 – 22°C).
Test room volume: 32m³

- **Carriers placement** :

The carriers were placed at a height of 1.31m, in a vertical position, towards the opposite side of the device

3-4 Interfering substance and culture media

-Interfering substance:	BSA fraction V at 0,3g/l (Batch N°345)
-Culture media:	EMEM (Batch N°2681)

4- Validations Protocol

4-1 Control of sensitivity of cells to virus

- Add one volume of solution S or PBS + one volume of cellular suspension at 2.10^5 cells/ml for one hour in water bath at 36°C±1°C
- The cells are centrifuged at 1600trs/min for 10 min and resuspended in culture media
- The virus is diluted from 1/10 to 1/10 on a 96-well microplate
- Add 100 µl of cell suspension treated (Solution S) or not treated (PBS control) to each well of the microplate
- Incubate for 72 hours

The difference of title reduction between cells treated by the solution S and cells treated by PBS shall be < 1 lg.

4-2 Control of efficiency for suppression of disinfectant activity

- Add 1 volume of BSA + 1 volume of virus suspension + 1 volume of solution S or distilled water
- Leave the mixture in the ice bath for 60 min at room temperature

5- Titration method

- Titrate the virus (method titration on cell in suspension) by following steps:
- Serial dilutions (1/10) are realized with culture medium in the glass tube
- Transfer 0,1 ml of each dilution into eight wells of a microplate plaque 96
- The last row of eight wells will receive 0,1 ml of culture medium (control untreated cells)
- Add 0,1 ml of cell suspension at 2.10^5cell/ml.
- Incubate for 72 hours at 36 ° C ± 1 ° C under 5% CO_2 ± 2%.
- The viral cytopathic effect is read by using an inverted microscope

The estimated of infectious unite is determined by method KARBER-SPAERMAN calculating the negative logarithm of 50% endpoint (lgDIC50) by the following formula:

lgDICT50 = negative logarithm of the highest concentration of virus - [(Sum of% affected to each dilution/100 - 0.5) X (lg dilution)]

6- Results

Virus suspension title assay: lgDICT50 = 8.50
No cytotoxicity was observed on the carrier without treatment which has been pretreated with the aerial disinfection system according to treatment.

	Degree of cytopathogenic effect (lgDICT50)	Logarithmic reduction
Sensitivity of cells to virus		
- With treatment (S1)		
Carrier 1	8.38	
Carrier 2	8.13	
Average	8.26	Difference <1 lg.
- Without traitement (S2)	8.63	
Carrier 1		
Efficiency for suppression of disinfectant activity		
- With treatment (D1)	7.0	
Carrier1	7.63	
Carrier 2	7.32	Difference <0,5 lg.
Average		
- Without traitement (D2)	7.50	
Carrier 1		
Test control		
Carrier1	7.13	
Carrier 2	7.0	
Average	7.1	
Assay		
Support 1	2.50	
Support 2	2.88	**4.27**
Support 3	3.13	
Average	2.83	

7- Conclusion

According to the conditions of test for the standard NF EN 17272 (April 2020), the couple device/product: NOCOSPRAY N° serial 172X731/NOCOLYSE Neutral 6% - Batch N° A281020N/1 for a use in medical area under clean condition, shows a virucidal activity against Human Coronavirus 229 E (log reduction≥4), after treatment at 5 mL/m^3 and 2 hours waiting time.

Chemila, spol. s r.o., Za Dráhou 4386/3, Hodonín 69501, Phone +420518340919, chemila@chemila.cz
Chemical and Microbiological Laboratory, Testing Laboratory No. 1273 certified by Czech Accreditation Institute according to ČSN EN ISO/IEC 17025:2018.

Copy No.: 3
Issue No.: 3

Test report No. S18-3/2020

DETERMINATION OF BACTERICIDAL (EN 13697:2015+A1:2019) ACTIVITY OF THE PRODUCT **NOCOLYSE**

Sample ID: S18/2020
Sample name: **Nocolyse**
Client: OXY'PHARM SARL, 829 rue Marcel Paul 94500 Champigny sur Marne, France
Producer: OXY'PHARM SARL, 829 rue Marcel Paul 94500 Champigny sur Marne, France
Sampling point: REMIX COM SRL, Ing. Teodorescu Street 37, Sector 6, Bucharest, Romania

Incoming date:
22.1.2020

Delivery date:
15.3.2022

Hodonín, 15.3.2022

Ing. Jana Šitrová, Head of Laboratory

The report may be reproduced only as a whole, in parts only upon written permission of the laboratory. The test results relate only to the samples stated in the Test Report. The Lab does not take any guarantee for the identity of samples not taken by the lab personnel.
The test report S18-3/2020 issued on 15.10.2020 was issued again on 8.2.2022 upon the client´s request for the correction of the interpretation.
The test report S18-3/2020 was issued again on 15.3.2022 upon the client´s request for the correction of the range of the tests and the client's name.

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: OXY'PHARM SARL, Champigny sur Marne, France

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 28.4. – 29.4.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

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

Identification of the sample:

Name of the product:	**Nocolyse**
Batch number:	061219N/1
Date of manufacture:	06/12/2019
Expiry date:	12/2021
Manufacturer:	OXY'PHARM SARL, 829 rue Marcel Paul 94500 Champigny sur Marne, France
Incoming date:	22.1.2020
Storage conditions:	stated by the manufacturer
Active ingredients:	CAS 7722-84-1 hydrogen peroxide 6%

Experimental conditions:	**Testing of disinfecting efficiency of chemical disinfecting and antiseptic agents on carriers** SOP-M-22-12 (EN 13697:2015+A1:2019)	
Period of analysis:	28.4. – 29.4.2020	
Test temperature:	18 °C ± 1 °C to 25 °C ± 1 °C	
Test method:	dilution neutralization method	
Neutralization medium:	Dey-Engley Neutralizing Broth M 1062	
Appearance of the product:	colourless liquid	
Test concentration:	100% (concentrated)	
Contact time:	5 min	
Interfering substances:	3.0 g/l BSA (dirty conditions)	
Test organisms:	*Escherichia coli*	ATCC 10536
	Pseudomonas aeruginosa	ATCC 15442
Incubation conditions:	37 °C ± 1 °C, 24 hours	

Test procedure:

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

Note:
Bactericidal activity – the capability of a product to produce a reduction in the number of viable bacterial cells of relevant organisms on carriers under defined conditions by at least a 4 lg reduction (10^4).
The drying time: 30 – 45 min

The standard:
EN 13697:2015+A1:2019 Chemical disinfectants and antiseptics – Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas – Test method and requirements without mechanical action (phase 2, step 2) April 2015 + August 2019

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: OXY'PHARM SARL, Champigny sur Marne, France

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 28.4. – 29.4.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

The Number of CFU in the tested product: 0 CFU/ml

1. Testing the efficacy of chemical disinfectant **Nocolyse** on carriers – bactericidal activity

Tab No. 1.1 Verification of methodology, dirty conditions

Test organisms	Test suspension N	Validation test	
		NT (Product conc.: 100%) Neutralization test	NC Neutralization control
Escherichia coli ATCC 10536	10^{-6}: >330, >330 10^{-7}: 39, 36 N: 6.97	10^{-3}: >330, >330 10^{-4}: 35, 33 NT: 6.53	10^{-3}: >330, >330 10^{-4}: 38, 36 NC: 6.57
Pseudomonas aeruginosa ATCC 15442	10^{-6}: >330, >330 10^{-7}: 42, 40 N: 7.01	10^{-3}: >330, >330 10^{-4}: 37, 47 NT: 6.62	10^{-3}: >330, >330 10^{-4}: 37, 38 NC: 6.57
Limit	$6.57 \leq \lg N \leq 7.10$	NT - Nc $\leq \pm 0.3$ lg	NC - Nc $\leq \pm 0.3$ lg

$N = \log_{10}[\{0.025 \cdot (x + x')\} /2 \cdot d]$ where x and x' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account
NC or NT $= \log_{10}[\{10 \cdot (y + y')\} /2 \cdot d]$
where y and y´ are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account

Tab No. 1.2 Testing the efficacy of chemical disinfectant **Nocolyse** on test strain, dirty conditions

Test organisms	Water control Nc	Test procedure Nd at concentrations / contact time (min)
		100% / 5
Escherichia coli ATCC 10536	10^{-3}: >330, >330 10^{-4}: 41, 39 Nc: 6.60 Nts: >100	10^{0}: <14, <14 Nd : < 2.15 Nts: 0 **R : ≥ 4.45**
Pseudomonas aeruginosa ATCC 15442	10^{-3}: >330, >330 10^{-4}: 55, 33 Nc: 6.64 Nts: >100	10^{0}: <14, <14 Nd : < 2.15 Nts: 0 **R : ≥ 4.49**
Limit	Nts: <100 CFU/ml for active concentration	

Nc or Nd $= \log_{10}[\{10 \cdot (a + a')\} /2 \cdot d]$
where a and a' are paired values for which the mean of the value falls between 14 and 330 colonies, d is the dilution factor for the dilution taken into account
Reduction R = Nc – Nd

2. Evaluation of bactericidal activity of the product **Nocolyse** on carriers

Tab No. 2.1 The efficacy of chemical disinfectant **Nocolyse** on test strains – bactericidal activity on carriers

Bactericidal and fungicidal activity of the product on carriers (EN 13697:2015+A1:2019)						
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations [%]	Interfering substances - conditions	R EN 13697:2015 +A1:2019	**R**
Escherichia coli ATCC 10536	18-25	5	100	dirty	≥ 4	**> 4**
Pseudomonas aeruginosa ATCC 15442	18-25	5	100	dirty	≥ 4	**> 4**

Reduction R= Nc – Nd

Prepared by: Mgr. Karolína Světlíková, Lab Technician

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

Sample ID: S18/2020
Rep No: 41
Sample name: **Nocolyse**
Sampled: by client
Sampling point: REMIX COM SRL, Bucharest, Romania
Client: OXY'PHARM SARL, Champigny sur Marne, France

Sampling date: 20.1.2020
Sample delivered: 22.1.2020
Testing date: 28.4. – 29.4.2020
Delivered amount: 2 x 1 l
Batch No: 061219N/1

Interpretation:
Results of tests are in Tabs.
According to EN 13697:2015+A1:2019 the tested concentrated product **Nocolyse,** batch No. 061219N/1, in the contact time 5 min under dirty conditions at temperature 18 °C ± 1 °C to 25 °C ± 1 °C by the dilution neutralization method **decreased** on carriers (stainless steel discs) the number of viable bacterial cells of *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442 10541by at least a 4 lg reduction.

Conclusion:
The product **Nocolyse** is capable of reducing the number of viable bacterial cells of the relevant organisms *Escherichia coli* and *Pseudomonas aeruginosa* under defined conditions (EN 13697:2015+A1:2019 – concentrated, 5 min, dirty conditions, 18 °C – 25 °C) to the declared values, and consequently, can be called bactericidal on *Escherichia coli* and *Pseudomonas aeruginosa* on carriers.
The test report S18-3/2020 issued on 15.10.2020 was issued again on 8.2.2022 upon the client´s request for the correction of the interpretation.
The test report S18-3/2020 was issued again on 15.3.2022 upon the client´s request for the correction of the range of the tests and the client's name.

15.3.2022, Hodonín

Approved by: Ing. Barbora Stoklásková, Leader of Study

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GĂMAN RAMONA-CRISTINA
Autorizație MINISTERUL JUSTIȚIEI

Traducere din limba engleză

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Laborator de chimie și microbiologie, Laborator de testare nr. 1273 certificat de către Institutul de Acreditare Ceh conform CSN EN ISO/IEC 17025:2018.

Exemplar nr. 3
Ediție nr. 3

Raport de Testare Nr. S18-3/2020

STABILIREA ACTIVITĂȚII BACTERICIDE (EN 13697:2015+A1:2019)
A PRODUSULUI **NOCOLYSE**

Nr. Identificare probă: S18/2020
Denumire probă: **Nocolyse**
Client: OXY'PHARM SARL, 829 Rue Marcel Paul, 94500 Champigny S/Mame, Franța
Producător: OXY'PHARM, 829 Rue Marcel Paul, 94500 Champigny S/Mame, Franța
Punct de prelevare probe: SC REMIX COM SRL, strada Ing. Teodorescu nr. 37, sector 6, București, România

Data intrării: 22.1.2020 Data livrării: 15.3.2022

Hodonin, 15.3.2022

Ștampilă Laborator de testare certificat de către Institutul de Acreditare Ceh Chemila, spol s.r.o. Za Drahou 4386/3, Hodonin 69501

Ing. Jana Slitrova, Șef laborator
Semnătură indescifrabilă

Raportul de testare poate fi reprodus numai în întregime, pe bucăți doar cu permisiunea scrisă a laboratorului. Rezultatele testelor se referă numai la probele menționate în Raportul de testare. Laboratorul nu acordă nicio garanție pentru identitatea probelor neprelevate de personalul laboratorului.
Raportul de testare S18-3/2020, emis la data de 15.10.2020, a fost emis din nou la data de 8.2.2022 la solicitarea clientului, pentru corectarea interpretării. Raportul de testare S18-3/2020 a fost emis

din nou la data de 15.3.2022 la solicitarea clientului, pentru corectarea intervalului de teste și a denumirii clientului.

Descriere: *Testarea eficacității dezinfectanților chimici și antisepticelor*

Nr. identificare probă: S18/2020	Dată prelevare: 20.1.2020
Nr. înregistrare: 41	Probă livrată: 22.1.2022
Denumire probă: **Nocolyse**	Dată testare: 28.4 – 29.4.2020
Prelevat: de către client	Cantitate livrată: 2 x 1 l
Punct de prelevare probe: SC REMIX COM SRL, strada Ing. Teodorescu nr. 37, sector 6, București, România	Nt. lot: 061219N/1
Client: OXY'PHARM SARL, 829 Rue Marcel Paul, 94500 Champigny S/Mame, Franța	Pagina:2

Obiectul testării:
Stabilirea activității bactericide a produsului.

Identificarea probei:

Denumirea produsului:	**Nocolyse**
Numărul lotului:	061219N/1
Data producției:	06/12/2019
Data expirării:	12/2021
Producător:	OXY'PHARM SARL, 829 Rue Marcel Paul, 94500 Champigny S/Mame, Franța
Data intrării:	22.1.2020
Condiții de depozitare:	indicate de către producător
Ingrediente active:	CAS 7722-84-1 peroxid de hidrogen 6%

Condiții experimentale:	**Testarea eficienței de dezinfectare a agenților chimici dezinfectanți și antiseptici pe agenți microbieni** SOP-M-22-12 (EN 13697:2015+A 1:2019)
Perioada analizei:	28.4.-29.4.2020
Temperatura de testare:	18°C± 1 °C până la 25 °C ± 1 °C
Metoda de testare:	metoda neutralizării prin diluție
Mediul de neutralizare:	mediu de cultură neutralizare Dey-Engley M 1062
Aspectul produsului:	lichid incolor
Concentrație de testare:	100% (concentrat)
Perioada de contact:	5 min
Substanțe de interferență:	BSA (albumină serică bovină) 3,0 g/l (condiții de murdărie)

Organisme de testare: *Escherichia coli* ATCC 10536
Pseudomonas aeruginosa ATCC 15442
Condiții de incubare: 37 °C ± 1 °C, 24 ore

Procedura de testare:

1. Prepararea suspensiei de testare
2. Prepararea soluțiilor de testare a produsului
3. Testarea cantitativă a agentului microbian
4. Incubare și calcul
5. Exprimarea și interpretarea rezultatelor

Notă:
Activitate bactericidă - capacitatea unui produs de a realiza o reducere a numărului de celule bacteriene viabile ale organismelor relevante pe agenți microbieni în condiții definite cu o reducere de cel puțin 4 lg (10^4).
Timp de uscare: 30 - 45 min

Standard:
EN 13697:2015+A1 :2019 Dezinfectanți chimici și antiseptice - Testarea cantitativă a suprafețelor neporoase pentru evaluarea activității bactericide și/sau fungicide a dezinfectanților chimici utilizați în domeniul alimentar, industrial, casnic și instituțional - Metodă de testare și cerințe fără acțiune mecanică (etapa 2, pasul 2) aprilie 2015 + august 2019

Numărul de CFU din produsul de testare: 0 CFU/ml

1. Testarea eficacității dezinfectantului chimic **Nocolyse** asupra agenților microbieni - activitate bactericidă

Tab nr. 1.1. Verificarea metodologiei, condiții de murdărie

Organisme de testare	Testarea suspensiei N	Test de validare	
		NT (Conc. Produs: 100%) Testare neutralizare	NC Control neutralizare
Escherichia coli ATCC 10536	10^{-6}: >330, >330 10^{-7}: 39, 36 N: 6,97	10^{-3}: >330, >330 10^{-4}: 35, 33 N: 6,53	10^{-3}: >330, >330 10^{-4}: 38, 36 N: 6,57
Pseudomonas aeruginosa ATCC 15442	10^{-6}: >330, >330 10^{-7}: 42, 40 N: 7,01	10^{-3}: >330, >330 10^{-4}: 37, 47 N: 6,62	10^{-3}: >330, >330 10^{-4}: 37, 38 N: 6,57
Limită	$6,57 \leq \lg N \leq 7,10$	NT - Nc $\leq \pm$ 0.3 lg	NC - Nc $\leq \pm$ 0,3 lg

$N = \log_{10} [\{0,025 \bullet (x + x')\} /2 \bullet d]$ unde x și x' sunt valori pereche pentru care media valorii se încadrează între 14 și 330 de colonii, d este factorul de diluție pentru diluția luată în considerare
$NC \text{ sau } NT = \log_{10} [\{10 \bullet (y + y')\} /2 \bullet d]$
unde y și y' sunt valori pereche pentru care media valorii se încadrează între 14 și 330 de colonii, d este factorul de diluție pentru diluția luată în considerare

Tab nr. 1.2. Testarea eficacității dezinfectantului chimic **Nocolyse** pe tulpina de testare, condiții de murdărie

Organisme de testare	Control apă Nc	Procedura de testare Nd la concentrații / perioada de contact (min)
		100% / 5
Escherichia coli ATCC 10536	10^{-3}: >330, >330 10^{-4}: 41, 39 Nc: 6,60 Nts: >100	10^{0}: <14, <14 Nd : < 2,15 Nts: 0 **R: ≥ 4,45**
Pseudomonas aeruginosa ATCC 15442	10^{-3}: >330, >330 10^{-4}: 55, 33 Nc: 6,64 Nts: >100	10^{0}: <14, <14 Nd : < 2,15 Nts: 0 **R: ≥ 4,49**
Limită	Nts: < 100 CFU/ml pentru concentrație activă	

$Nc \text{ sau } Nd = \log_{10} [\{10 \bullet (a + a')\} /2 \bullet d]$
unde a și a' sunt valori pereche pentru care media valorii se situează între 14 și 330 de colonii, d este factorul de diluție pentru diluția luată în considerare
Reducere R = Nc – Nd

2. Evaluarea activității bactericide a produsului **Nocolyse** pe agenți microbieni

Tab nr. 2.1 Eficacitatea dezinfectantului chimic **Nocolyse** pe tulpinile de testare - activitatea bactericidă pe agenții microbieni

Activitate bactericidă și fungicidă a produsului pe agenții microbieni (EN 13697:2015+A1:2019)						
Tulpină	Temperatura de testare [°C]	Perioada de contact [min]	Concentrații testare produs [%]	Substanțe de interferență - condiții	R EN 13697:2015 +A1:2019	R
Escherichia coli ATCC 10536	18-25	5	100	murdărie	>4	>4
Pseudomonas aeruginosa ATCC 15442	18-25	5	100	murdărie	>4	> 4

Reducere R= Nc – Nd

Pregătit de către: Mgr. Karolina Svetlikova, Tehnician laborator

Interpretare:
Rezultatele testelor sunt în tabele.
Conform EN 13697:2015+A1:201, produsul concentrat testat **Nocolyse**, lotul nr. 061219N/1, în perioada de contact de 5 min în condiții de murdărie la temperatura de 18 °C ± 1 °C până la 25 °C ± 1 °C prin metoda de neutralizare a diluției a **scăzut** pe agenții microbieni (discuri din oțel inoxidabil) numărul de celule bacteriene viabile ale *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442 10541, cu cel puțin o reducere de 4 lg.

Concluzie:
Produsul **Nocolyse** este capabil să reducă numărul de celule bacteriene viabile ale organismelor relevante *Escherichia coli* și *Pseudomonas aeruginosa* în condiții definite (EN 13697:2015+A1:2019 - concentrat, 5 min, condiții de murdărie, 18 °C - 25 °C) la valorile declarate și, în consecință, poate fi numit bactericid pe *Escherichia coli* și *Pseudomonas aeruginosa* pe agenții microbieni.
Raportul de testare S18-3/2020, emis în data de 15.10.2020, a fost emis din nou în data de 8.2.2022 la solicitarea clientului, pentru corectarea interpretării.
Raportul de testare S18-3/2020 a fost emis din nou la data de 15.3.2022 la solicitarea clientului, pentru corectarea intervalului de teste și a denumirii clientului.

15.3.2022, Hodonin

Aprobat de: Ing. Barbora Stoklaskova, Coordonator studiu

Ștampilă Laborator de testare certificat de către
Institutul de Acreditare Ceh
Chemila, spol s.r.o.
Za Drahou 4386/3, Hodonin 69501

*Subsemnata **GĂMAN RAMONA CRISTINA** interpret si traducător autorizat pentru limbile străine engleză şi italiană, în temeiul autorizaţiei nr. **7332** din **2002** eliberată de Ministerul Justiţiei din România, certific exactitatea traducerii efectuate din limba engleză în limba română.*

Traducător,
Ramona-Cristina Găman