





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 17025:2005.

Copy No.: 1 Issue No.: 1

Test report No. S103/2019

DETERMINATION OF BACTERICIDAL (EN 16615:2015) ACTIVITY OF THE PRODUCT **F173**

Sample ID: S103/2019

Sample name: F173

Client: Christeyn France S.A., 31, Rue de la Maladrie, 44124 Vertou, France Producer: Christeyn France S.A., 31, Rue de la Maladrie, 44124 Vertou, France Sampling point: Christeyn France S.A., 31, Rue de la Maladrie, 44124 Vertou, France

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Incoming date: 5.3.2019

Delivery date: 1.7.2019

Hodonín, 1.7.2019



Ing. Jana Šlitrová, Head of Laboratory

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Sample ID: S103/2019

Rep No: 62 Sample name: F173 Sampled: by client

Sampling point: Christeyn France S.A., Vertou

Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 26.2.2019 Sample delivered: 5.3.2019 Testing date: 30.4. - 2.5.2019Delivered amount: 150 ml

Batch No: 181221/0823-01

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Subject of testing:

Determination of bactericidal activity of the product.

Identification of the sample:

Name of the product:

F173

Batch number: Date of manufacture:

181221/0823-01 21/12/2018

Expiry date:

2021-11

Manufacturer:

Christeyn France S.A., 31, Rue de la Maladrie, 44124 Vertou, France

Incoming date: Storage conditions: 5.3.2019 5-30 °C

Active compounds and concentrations:

CAS 2372-82-9 N-(3-aminopropyl)-N-dodecylpropane-1,3 diamine 5.5 %

CAS 7173-51-5 Didecyldimethylammonium chloride 3.5 %

Testing of disinfecting efficiency of chemical disinfecting and Experimental conditions:

> antiseptic agents on carriers SOP-M-19-00 (EN 16615:2015)

Period of analysis:

30.4. - 2.5.2019 $20 \, ^{\circ}\text{C} \pm 2.5 \, ^{\circ}\text{C}$ $20 \, ^{\circ}\text{C} \pm 1 \, ^{\circ}\text{C}$

Lab temperature: Temperature of media:

Test method:

dilution neutralization method

Neutralization medium:

Dey-Engley Neutralizing Broth M 1062

Product diluent:

hard water

Appearance of the product:

light yellow liquid hard water + polysorbate 80

Test concentration:

0.1%, 0.25%

Water control: Contact time:

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

ATCC 10541 Enterococcus hirae

Incubation conditions:

 $37 \, ^{\circ}\text{C} \pm 1 \, ^{\circ}\text{C}, 24 \, (48) \text{ hours}$

Test surface:

PVC with PUR coating, width 2.5 mm, 20 cm x 50 cm. The surface is cleaned by 70% n-propanol. After drying draw 4 squares 5 cm x 5 cm 5 cm apart, mark them as test fields 1 to 4. The drying controls D_{C0} and D_{Ct} are performed on smaller surface (7 cm x 13 cm, 2 squares 5

cm x 5 cm).

Wipe:

17.5 cm x 28 cm, 55% cellulose, 45% polyethylenterephtalate (PET), the wipe is used only once. 30 minutes before testing put the wipe in Petri dish with 16 ml of the product solution. The wet wipe is weighed

before and after testing.

Test weight: Tampons:

granite, lenght 11.9 cm, width 8.2 cm, height 8.4 cm, weight 2.4 kg sterile, length 150 mm, disposable, tip made of pure cotton without compounds inhibiting or supporting the effect of product solution or

growth of microorganisms, producer F.L. Medical

Parafilm® M, 10.2 cm x 38 m, producer Brand Parafilm:

disposable, protecting the horisontal surface and vertical surfaces

before contamination during wiping.

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Sampling date: 26.2.2019 Sample delivered: 5.3.2019 Testing date: 30.4. – 2.5.2019 Delivered amount: 150 ml Batch No: 181221/0823-01

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Test procedure:

- 1. Preparation of the test suspension
- 2. Determination of CFU in the test suspension
- 3. Quantitative test on carriers according to EN 16615:2015
- 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 on nonporous surface in the field 1 by at least a 5 lg reduction (10^5). $R = D_{Ct}/N_a$ or $lg R = lg D_{Ct} - lg N_a$ the reduction in viability, the drying time: 15 - 20 min

The standard:

EN 16615:2015 Chemical disinfectants and antiseptics – Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4-field test) – Test method and requirements (phase 2, step 2) April 2015

EN ISO 4833-1 Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 degrees C by the pour plate technique, September 2013

The Number of CFU in the tested product F173 (SOP-M-07-00 (EN ISO 4833-1)): 0 CFU/ml

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Delivered amount: 150 ml Batch No: 181221/0823-01

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1. Testing the efficacy of chemical disinfectant **F173** on *Pseudomonas aeruginosa* ATCC 15442 on non-porous surfaces

Tab No. 1.1 Verification of methodology, temperature 20°C, dirty conditions

Valida	Validation of suspension (N _{V0})		Neutra	lizer toxicity con	trol (B)	Method validation (C), product conc. 0.25			
V _{cl}	60	Φ − 52	V _{c1}	72	A 51.5	V _{c1}	38	± 50.5	
V_{c2}	44	$\Phi_{\text{Nvo}} = 52$	V _{c2} 31		$\Phi_{\rm B} = 51.5$	V _{c2}	67	$\Phi_{\rm C}=52.5$	
$30 \le \Phi_{\text{Nvo}} \le 160$		$\Phi_{\rm B} \ge 0.5 \; \Phi_{\rm Nvo}$			$\Phi_{\rm C} \ge 0.5 \; \Phi_{\rm Nyo}$				
X	yes	no	X	yes	no	X	yes	no	

Tab No. 1.2 Test suspension

Test suspension N	Dilution	V_{cl}	V_{cl}		Test suspension N_0 $N_0 = N/20$, $\log N_0 = 8.38$			
$\Phi = 48 \times 10^8 = \lg 9.68$	10-7	> 330	> 330					
$9.17 \le \lg N \le 9.70$	10-8	62	34		$7.88 \le \lg N_0 \le 8.40$			
				х	ves	no		

Tab No. 1.2.1 Drying in time 0

Drying control (D _{C0})	Dilution	Vci	Vcl		1. D. 1. (# 5 105 TO	
	10 ⁻⁵	168	175		$lg D_{C0} = lg (\Phi \times 5 \times 10^5) = 7.93$		
	10-6	18	17		6.88 ≤	$\lgD_{C0} \le 8.40$	
				х	ves	no	

Tab No. 1.2.2 Drying in time t

Drying control (D _{Ct})	Dilution	Vcl	Vcl		1 D 1 (a 5 105) 7.07
	10-5	152	143		$\lg D_{Ct} = \lg (\Phi \times 5 \times 10^5) = 7.87$	
	10-6	16	14		$6.88 \le 1 \text{g D}_{\text{Ct}} \le 8.40$	$\lg D_{Ct} \le 8.40$
				х	ves	no

Tab No. 1.3.1 Test with water N_w – the effect of water (Wipe with hard water + polysorbate 80) on *Pseudomonas aeruginosa* ATCC 15442 on non-porous surfaces, dirty conditions

Field / contact time Dilution after test Nw requirement procedure $(\Phi \times 5)$ (min) >10 cfu/25 cm² 2/15 10^{0} 3 15 ves 10^{0} 3 / 15 3 15 yes 10^{0} 4/15 4 20 yes

Tab No. 1.3.2.1 Test – the effect of F173 (Wipe with product solution) on *Pseudomonas aeruginosa* ATCC 15442 on non-porous surfaces, dirty conditions, field 2-4

Test concentration (%) /contact time (min) /conditions / field	Dilution after test procedure	V_c	$N_a = (\Phi \times 5)$	N _a requirement <50 cfu/25 cm ²
0.25/15/dirty/2	10°	0	<14	yes
0.25/15/dirty/3	10°	0	<14	yes
0.25/15/dirty/4	10 ⁰	0	<14	yes

Tab No. 1.3.2.2 Test – the effect of F173 (Wipe with product solution) on *Pseudomonas aeruginosa* ATCC 15442

on non-porous surfaces, dirty conditions, field 2-4

Test concentration (%) /contact time (min) /conditions / field	Dilution after test procedure	V_c	$N_a = (\Phi \times 5)$	N _a requirement <50 cfu/25 cm ²
0.1/15/dirty/2	10°	3	15	yes
0.1/15/dirty/3	10 ⁰	0	<14	yes
0.1/15/dirty/4	10°	0	<14	yes

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Sample name: F173
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Sampling date: 26.2.2019 Sample delivered: 5.3.2019 Testing date: 30.4. – 2.5.2019 Delivered amount: 150 ml

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Tab No. 1.3.3 Test – the effect of F173 (Wipe with product solution) on *Pseudomonas aeruginosa* ATCC 15442 on non-porous surfaces, dirty conditions, field 1

Test concentration (%) /contact time (min) /conditions / field	Dilution after test procedure	V_{c1}	V_{c2}	lg N _a (Φ x 5)	
0.25/15/dirty/1	10°	<14	<14	<1.85	≥ 6.02
0.1/15/dirty/1	10-1	21	31	3.11	4.76

Tab No. 1.4 Test – weight of wipes before and after testing

Weight of wipes	Weight before testing (g)	Weight after testing (g)	Difference (g)
F173 (Wipe with 0.25% solution)	18.9	17.9	1.0
F173 (Wipe with 0.1% solution)	19.1	17.7	1.4
Wipe with hard water + polysorbate 80	19.1	17.9	1.2

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 in the bacterial test suspension, N_{V0} = the number of cfu/ml in the bacterial test suspension for validation, N_a = the number of viable bacterial cells per ml in the test mixture, A, B, C = the number of viable bacterial cells per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation $R = D_{Ct}/N_a$ or $lg R = lg D_{Ct} - lg N_a$ the reduction in viability

Sample ID: S103/2019

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Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 26.2.2019 Sample delivered: 5.3.2019 Testing date: 30.4 - 2.5.2019

Testing date: 30.4. – 2.5.2019 Delivered amount: 150 ml Batch No: 181221/0823-01

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2. Testing the efficacy of chemical disinfectant F173 on Staphylococcus aureus ATCC 6538 on non-porous surfaces

Tab No. 2.1 Verification of methodology, temperature 20°C, dirty conditions

Valid	ation of suspension	on (N _{V0})	Neutralizer toxicity control (B)		trol (B)	Method validation (C), product conc. 0.25			
V _{c1}	37	Φ - 41 F	V _{c1}	33	A 265	V _{c1}	33	. A. 21	
V_{c2}	46	$\Phi_{\text{Nvo}} = 41.5$	V_{c2}	$\Phi_{\rm B} = 36.5$	V_{c2}	29	$\Phi_{\rm C} = 31$		
30 ≤ 0	$\Phi_{\text{Nvo}} \leq 160$		$\Phi_{\rm B} \ge 0$.5 Φ _{Nvo}		$\Phi_{\rm C} \ge 0$.	.5 Φ _{Nvo}		
X	yes	no	x	yes	no	X	yes	no	

Tab No. 2.2 Test suspension

Test suspension N	Dilution	Dilution V_{c1} V_{c1}		Test suspension N ₀			
$\Phi = 42.5 \times 10^8 = \lg 9.63$	10-7	> 330	> 330	$N_0 = N/20$, $\log N_0 = 8.33$			
$9.17 \le \lg N \le 9.70$	10-8	48	37	$7.88 \le \lg N_0 \le 8.40$			

Tab No. 2.2.1 Drying in time 0

	Dilution	Vc1	V_{c1}		1- D 1- 6	5 . <i>C</i>	105) 7.00
Drying control (D _{C0})	10-4	> 330	> 330		$\lg D_{C0} = \lg (\Phi \times 5 \times 10^5) = 7.80$		
	10 ⁻⁵	132	119		$6.88 \le \lg D_{C0} \le 8.40$		
				х	ves		no

Tab No. 2.2.2 Drying in time t

Drying control (D _{Ct})	Dilution	V_{cl}	V_{cl}		1- D -1- (6	h	05) - 7.75
	10-4	> 330	> 330		$lg D_{Ct} = lg (\Phi \times 5 \times 10^{5}) = 7.75$ 6.88 \le lg D_{Ct} \le 8.40		
	10-5	109	118				8.40
				х	ves		no

Tab No. 2.3.1 Test with water N_w – the effect of water (Wipe with hard water + polysorbate 80) on *Staphylococcus*

aureus ATCC 6538 on non-porous surfaces, dirty conditions

Field / contact time (min)	Dilution after test procedure	V_c	$N_W = (\Phi \times 5)$	N _w requirement >10 cfu/25 cm ²
2 / 15	10°	5	25	yes
3 / 15	10°	5	25	yes
4 / 15	10 ⁰	8	40	yes

Tab No. 2.3.2.1 Test – the effect of F173 (Wipe with product solution) on Staphylococcus aureus ATCC 6538 on

non-porous surfaces, dirty conditions, field 2-4

Test concentration (%) /contact time (min) /conditions / field	Dilution after test procedure	V_c	$N_a = (\Phi \times 5)$	N _a requirement <50 cfu/25 cm ²
0.25/15/dirty/2	10°	1	<14	yes
0.25/15/dirty/3	10°	3	15	yes
0.25/15/dirty/4	10°	3	15	yes

Tab No. 2.3.2.2 Test – the effect of **F173** (Wipe with product solution) on *Staphylococcus aureus* ATCC 6538 on non-porous surfaces, dirty conditions, field 2-4

Dilution after test Test concentration (%) Na requirement $N_a =$ /contact time (min) $(\Phi \times 5)$ <50 cfu/25 cm² procedure /conditions / field 0.1/15/dirty/2 10^{0} 0 <14 ves 10^{0} 0.1/15/dirty/3 2 <14 yes 0.1/15/dirty/4 10^{0} 0 <14 yes

Sample ID: S103/2019

Rep No: 62

Sample name: **F173** Sampled: by client

Sampling point: Christeyn France S.A., Vertou

Client: Christeyn France S.A., 31, Rue de la Maladrie, Vertou

Sampling date: 26.2.2019 Sample delivered: 5.3.2019 Testing date: 30.4. – 2.5.2019

Delivered amount: 150 ml Batch No: 181221/0823-01

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Tab No. 2.3.3 Test – the effect of **F173** (Wipe with product solution) on *Staphylococcus aureus* ATCC 6538 on non-porous surfaces, dirty conditions, field 1

Test concentration (%) Dilution after V_{c2} lg N_a (Φ x 5) lg R /contact time (min) test procedure $(lg D_{Ct} = 7.75)$ /conditions / field 0.25/15/dirty/1 10⁰ 17 18 1.94 5.81 0.1/15/dirty/1 10° 78 74 2.58 5.17

Tab No. 2.4 Test - weight of wipes before and after testing

Weight of wipes	Weight before testing (g)	Weight after testing (g)	Difference (g)
F173 (Wipe with 0.25% solution)	19.2	17.9	1.3
F173 (Wipe with 0.1% solution)	19.2	18.0	1.2
Wipe with hard water + polysorbate 80	19.3	18.3	1.0

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 in the bacterial test suspension, N_{V0} = the number of cfu/ml in the bacterial test suspension for validation, N_a = the number of viable bacterial cells per ml in the test mixture, A, B, C = the number of viable bacterial cells per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation $R = D_{Ct}/N_a$ or $lg R = lg D_{Ct} - lg N_a$ the reduction in viability

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3. Testing the efficacy of chemical disinfectant F173 on Enterococcus hirae ATCC 10541 on non-porous surfaces

Valida	ation of suspension	n (N _{V0})		temperature 20° alizer toxicity cont			d validation (C).	product conc. 0.25%
¥7	1 40							product conc. 0.2570
V _{el}	49	$\Phi_{\text{Nyo}} = 45$	V_{c1}	46	A 12	V _{c1}	26	
V _{c2}	41	ΨNvo — ¬J	V _{c2}	V_{c2} 40 $\Phi_B = 43$		V _{c2} 50	$\Phi_{\rm C} = 38$	
30 ≤ Φ	P _{Nvo} ≤ 160		$\Phi_{\rm B} \geq 0$	0.5 Φ _{Nvo}		$\Phi_{\rm C} > 0$).5 Φ _{Nyo}	
X	yes	no	X	yes	no	X	ves	no

Tab No. 3.2 Test suspension

Test suspension N	Dilution	V_{c1}	V_{c1}		Test suspension N ₀		
$\Phi = 45.5 \times 10^8 = \lg 9.66$	10-7	> 330	> 330		$N_0 = N/20$, $\log N_0 = 8.36$ $7.88 \le \log N_0 \le 8.40$		
$9.17 \le \lg N \le 9.70$	10-8	42	49				
	10	72	49	x	7.88 ≤	$\frac{\lg N_0 \le 8.40}{n_0}$	

Tab No. 3.2.1 Drying in time 0

Drying control (D _{C0})	Dilution	V_{c1}	V_{cl}			
	10-4	> 330	> 330		$\lg D_{C0} = \lg (\Phi \times 5 \times 10^5) = 7.77$	
	10-5	110	126		6.88 ≤	$lg D_{C0} \le 8.40$
				х	ves	no

Tab No. 3.2.2 Drying in time t

	Dilution	V_{cl}	V _{cl}		1.0		
Drying control (D _{Ct})	10-4	> 330	> 330			$\Phi \times 5 \times 10^5 = 7.67$	
	10-5	99	88		$6.88 \le \lg D_{Ct} \le 8.40$		
				х	yes	no	

Tab No. 3.3.1 Test with water N_w - the effect of water (Wipe with hard water + polysorbate 80) on *Enterococcus*

hirae ATCC 10541 on non-porous surfaces, dirty conditions

Field / contact time (min)	Dilution after test procedure	V_{c}	$N_W = (\Phi \times 5)$	N _w requirement >10 cfu/25 cm ²
2 / 15	10°	18	90	ves
3 / 15	10 ⁰	72	360	ves
4 / 15	10°	18	90	ves

Tab No. 3.3.2.1 Test – the effect of **F173** (Wipe with product solution) on *Enterococcus hirae* ATCC 10541 on non-porous surfaces, dirty conditions, field 2-4

Test concentration (%) /contact time (min) /conditions / field	Dilution after test procedure	V _c	$N_a = (\Phi \times 5)$	N _a requirement <50 cfu/25 cm ²
0.25/15/dirty/2	10 ⁰	2	<14	ves
0.25/15/dirty/3	10 ⁰	3	15	ves
0.25/15/dirty/4	10 ⁰	0	<14	ves

Tab No. 3.3.2.2 Test – the effect of **F173** (Wipe with product solution) on *Enterococcus hirae* ATCC 10541 on non-porous surfaces, dirty conditions, field 2-4

Test concentration (%) /contact time (min) /conditions / field	Dilution after test procedure	V_{c}	$N_a = (\Phi \times 5)$	N _a requirement <50 cfu/25 cm ²
0.1/15/dirty/2	10 ⁰	7	35	ves
0.1/15/dirty/3	10 ⁰	0	<14	ves
0.1/15/dirty/4	10^{0}	0	<14	ves

Sample ID: S103/2019

Rep No: 62

Sample name: **F173** Sampled: by client

Sampling point: Christeyn France S.A., Vertou

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Sampling date: 26.2.2019 Sample delivered: 5.3.2019 Testing date: 30.4. – 2.5.2019 Delivered amount: 150 ml

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Tab No. 3.3.3 Test – the effect of F173 (Wipe with product solution) on *Enterococcus hirae* ATCC 10541 on non-porous surfaces, dirty conditions, field 1

porous burraces, unit	conditions, neit	1			
Test concentration (%) /contact time (min) /conditions / field	Dilution after test procedure	V_{cl}	$ m V_{c2}$	lg N _a (Φ x 5)	$ \frac{\lg R}{(\lg D_{Ct} = 7.67)} $
0.25/15/dirty/1	10 ⁰	<14	<14	<1.85	≥ 5.82
0.1/15/dirty/1	100	77	68	2.56	5.11

Tab No. 3.4 Test - weight of wipes before and after testing

Weight of wipes	Weight before testing (g)	Weight after testing (g)	Difference (g)
F173 (Wipe with 0.25% solution)	19.2	18.1	1.1
F173 (Wipe with 0.1% solution)	19.0	17.9	1.1
Wipe with hard water + polysorbate 80	19.4	18.1	1.3

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 in the bacterial test suspension, N_{V0} = the number of cfu/ml in the bacterial test suspension for validation, N_a = the number of viable bacterial cells per ml in the test mixture, A, B, C = the number of viable bacterial cells per ml in control tests (A – experimental conditions validation, B – neutralizer toxicity validation, C – method validation $R = D_{Ct}/N_a$ or $lg R = lg D_{Ct} - lg N_a$ the reduction in viability

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4. Evaluation of bactericidal activity of the product F173

Tab No. 4.1 The efficacy of chemical disinfectant **F173** on test strains – bactericidal activity on non-porous surfaces, dirty conditions, field 1

Bacter	ricidal and yeastic	cidal activity	of the product (EN 1	16615:2015)		
Strain	Test temperature [°C]	Contact time [min]	Product test concentrations	Interfering substances – conditions	lg R EN 16615:2015	lg R
Pseudomonas aeruginosa ATCC 15442	20	15	0.25	dirty	> 5	< 5
Staphylococcus aureus ATCC 6538	20	15	0.25	dirty	≥ 5	> 5
Enterococcus hirae ATCC 10541	20	15	0.25	dirty	> 5	> 5
Pseudomonas aeruginosa ATCC 15442	20	15	0.1	dirty	> 5	> 5
Staphylococcus aureus ATCC 6538	20	15	0.1	dirty	≥ 5	> 5
Enterococcus hirae ATCC 10541	20	15	0.1	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 in the test suspension, N_{V0} = the number of cfu/ml in the test suspension for validation, N_a = the number of bacteria and fungi per ml in the test mixture, A, B, C = the number of bacteria and fungi per ml in control tests (A - experimental conditions validation, B - neutralizer toxicity validation, C - method validation $R = D_{Ct}/N_a$ or $\log R = \log D_{Ct} - \log N_a$ the reduction in viability

Sample ID: S103/2019

Rep No: 62

Sample name: **F173** Sampled: by client

Sampling point: Christeyn France S.A., Vertou

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Sampling date: 26.2.2019 Sample delivered: 5.3.2019 Testing date: 30.4. – 2.5.2019 Delivered amount: 150 ml Batch No: 181221/0823-01

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Interpretation:

Results of tests are in Tabs.

According to EN 16615:2015 the tested product F173, batch No. 181221/0823-01, in the concentration 0.25%, diluted in hard water (soaked wipe) and in the contact time 15 min under dirty conditions at temperature 20 °C ± 2.5 °C by the dilution neutralization method **decreased** on non-porous surfaces on field 1 the number of viable bacterial cells of *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541 by at least a 5 lg reduction.

Conclusion:

The product F173 is capable of reducing the number of viable bacterial cells of the relevant organisms on non-porous surfaces under defined conditions to the declared values and, consequently, may be called bactericidal.

1.7.2019, Hodonín

Ing. Barbora Stoklásková, Leader of Study

Chemilla, spet. s.r.o. Za Dráhov 4386/3

695 01 Hedonín