



Toulouse, February 5th 2013

TEST REPORT N°13-572

STUDY 12-1401

NF EN 13697 (November 2001)
QUANTITATIVE NON-POROUS SURFACE TEST FOR THE
EVALUATION OF BACTERICIDAL ACTIVITY OF CHEMICAL
DISINFECTANTS USED IN FOOD, INDUSTRIAL, DOMESTIC
AND INSTITUTIONAL AREAS
Dirty conditions
(Test method and requirements without mechanical action)

Client

CVE sarl
ZI de Pechnauquié
31340 VILLEMUR SUR TARN

Assay Laboratory

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Chemical disinfectants and antiseptics
Quantitative non-porous surface test for the evaluation of bactericidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas
ACCORDING TO STANDARD EN 13697 (November 2001) - Dirty conditions
Test method and requirements without mechanical action

I - IDENTIFICATION OF ASSAY LABORATORY

FONDEREPHAR

Faculté des Sciences Pharmaceutiques
35 Chemin des Maraîchers
31062 Toulouse cedex 9

II - IDENTIFICATION OF TEST PRODUCT

Product name.	Ciel Vert 300B
Batch.	F0529.12
Date of receipt.	Jul-06-2012
Internal Code.	12-1401-1
Active Substance.	Not defined
Promotor.	CVE
Period of testing.	November 2012 - February 2013
Storage conditions.	Room temperature

III - TEST

Dilution neutralization method

Count per ml : 1 or 2 for counting of inoculum (N)

Neutralizer. Tween 80 (10%), Lecithin (2%), Saponin (2%), Sodium thiosulfate (0.5%), trypticase-soja broth (Batches 842, 843 and 849)

Appearance of product dilution: clear, except for 10% dilution, slightly opalescent

Test surface: stainless steel discs 1.4301 (EN 10088-1), 2 cm in diameter, grade 2B according to the requirements of EN 10088-2, bilateral finish and 1.2 or 1.5 mm thick .

IV - EXPERIMENTAL CONDITIONS

Product diluent. Hard water (hardness < 300 mg/kg in the test tube)

Concentrations of the product: 10%, 5% et 0,1% (V/V)

Interfering substance. 3 g/l bovine albumin (dirty conditions) (Batch 88)

Obligatory Conditions

Test-organism *Staphylococcus aureus* CIP 4.83 (ATCC 6538)
Pseudomonas aeruginosa CIP 103467 (ATCC 15442)
Enterococcus hirae CIP 5855 (ATCC 10541)
Escherichia coli CIP 54127 (ATCC 10536)

Test temperature : between 18 and 25 ° C \pm 1 ° C

Contact time : 5 minutes \pm 10 seconds

Incubation temperature. 36 \pm 1°C

No precipitate during the test procedure (test mixtures were homogeneous), except for the concentration 5%, an precipitate and an opalescence.

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V - TEST RESULTS

STAPHYLOCOCCUS AUREUS : Ciel Vert 300B - Batch F0529.12 - Assay 1

VALIDATION				TEST SUSPENSION		TEST			
	Control water	Neutralizer control	Method validation 10 %	N		Contact time	Concentration of product % (V/V)		
	Nc	NC	NT	10 ⁻⁶	10 ⁻⁷		10%	5%	0,1%
X	10 ⁻³ >300 - >300 10 ⁻⁴ 82 - 82 10 ⁻⁵ 16 - 11 10 ⁻⁶ 1 - 0 Nts : > 300	10 ⁻³ >300 - >300 10 ⁻⁴ 92 - 101 10 ⁻⁵ 8 - 5	10 ⁻³ >300 - >300 10 ⁻⁴ 78 - 77 10 ⁻⁵ 10 - 7	143+150 151+160	32 - 30	5 min	10 ⁰ 0 - 0 10 ⁻¹ 0 - 0 10 ⁻² 0 - 0 Nts : 0	10 ⁰ 25 - 23 10 ⁻¹ 1 - 1 10 ⁻² 0 - 0 Nts : 1	10 ⁰ >300->300 10 ⁻¹ >300->300 10 ⁻² >300->300 Nts : >300
Log	6,91	6,98	6,89	X = 302,00.10 ⁶ N = 7,18		Nd	< 0,10	< 2,70	> 5,48
Validation criteria	N - Nc ≤ 2 log	N - NC ≤ 2 log	NC - NT ≤ +/- 0,3	1,5.10 ⁸ ≤ X ≤ 5.10 ⁸ (X = UFC/ML)		ME =Nc - Nd	> 6,81	> 4,21	< 1,43

Date of the test: Nov/13/2012
 N = Count per ml (1 or 2 plates for N/10⁻⁶)

PSEUDOMONAS AERUGINOSA : BLUETOO : Ciel Vert 300B - Batch F0529.12 - Assay 1

VALIDATION				TEST SUSPENSION		TEST			
	Control water	Neutralizer control	Method validation 10 %	N		Contact time	Concentration of product % (V/V)		
	Nc	NC	NT	10 ⁻⁶	10 ⁻⁷		10%	5%	0,1%
X	10 ⁻² >300 - >300 10 ⁻³ 57 - 52 10 ⁻⁴ 10 - 13 10 ⁻⁵ 0 - 2 10 ⁻⁶ 0 - 0 Nts : 148	10 ⁻² 152 - 167 10 ⁻³ 17 - 20 10 ⁻⁴ 3 - 2 10 ⁻⁵ 0 - 0	10 ⁻² 163 - 128 10 ⁻³ 10 - 9 10 ⁻⁴ 3 - 1 10 ⁻⁵ 0 - 0	170+142 136+160	37 - 39	5 min	10 ⁰ 0 - 0 10 ⁻¹ 0 - 0 10 ⁻² 0 - 0 Nts : 1	10 ⁰ 1 - 0 10 ⁻¹ 0 - 0 10 ⁻² 0 - 0 Nts : 0	10 ⁰ >300->300 10 ⁻¹ >300->300 10 ⁻² 28 - 38 Nts : 1
Log	5,74	5,20	5,16	X = 304,00.10 ⁶ N = 7,18		Nd	< 0,10	< 0,10	> 4,70
Validation criteria	N - Nc ≤ 2 log	N - NC ≤ 2 log	NC - NT ≤ +/- 0,3	1,5.10 ⁸ ≤ X ≤ 5.10 ⁸ (X = UFC/ML)		ME = Nc - Nd	> 5,64	> 5,64	< 1,04

Date of the test: Nov/21/2012
 N = Count per ml (1 or 2 plates for N/10⁻⁶)

ENTEROCOCCUS HIRAE : Ciel Vert 300B - Batch F0529.12 - Assay 1

VALIDATION				TEST SUSPENSION		TEST			
	Control water	Neutralizer control	Method validation 10 %	N		Contact time	Concentration of product % (V/V)		
	Nc	NC	NT	10 ⁻⁶	10 ⁻⁷		10%	5%	0,1%
X	10 ⁻³ >300 - >300 10 ⁻⁴ 53 - 57 10 ⁻⁵ 8 - 6 10 ⁻⁶ 1 - 1 Nts : 28	10 ⁻³ >300 - >300 10 ⁻⁴ 42 - 53 10 ⁻⁵ 8 - 4	10 ⁻³ >300 - >300 10 ⁻⁴ 41 - 64 10 ⁻⁵ 4 - 4	107+100 91+109	25 - 22	5 min	10 ⁰ 2 - 2 10 ⁻¹ 0 - 0 10 ⁻² 0 - 0 Nts : 0	10 ⁰ 0 - 0 10 ⁻¹ 0 - 0 10 ⁻² 0 - 0 Nts : 0	10 ⁰ >300->300 10 ⁻¹ >300->300 10 ⁻² >300->300 Nts : >300
Log	6,74	6,68	6,72	X = 203,50.10 ⁶ N = 7,01		Nd	< 0,10	< 0,10	> 5,48
Validation criteria	N - Nc ≤ 2 log	N - NC ≤ 2 log	NC - NT ≤ +/- 0,3	1,5.10 ⁸ ≤ X ≤ 5.10 ⁸ (X = UFC/ML)		ME = Nc - Nd	> 6,64	> 6,64	< 1,26

Date of the test: Nov/13/2012
N = Count per ml (1 or 2 plates for N/10⁻⁶)

ESCHERICHIA COLI : Ciel Vert 300B - Batch F0529.12 - Assay 1

VALIDATION				TEST SUSPENSION		TEST			
	Control water	Neutralizer control	Method validation 10 %	N		Contact time	Concentration of product % (V/V)		
	Nc	NC	NT	10 ⁻⁶	10 ⁻⁷		10%	5%	0,1%
X	10 ⁻² >300 - >300 10 ⁻³ 53 - 61 10 ⁻⁴ 6 - 5 10 ⁻⁵ 0 - 1 10 ⁻⁶ 0 - 1 Nts : 6	10 ⁻² 296 - 289 10 ⁻³ 37 - 32 10 ⁻⁴ 0 - 7 10 ⁻⁵ 0 - 0	10 ⁻² >300 - >300 10 ⁻³ 52 - 52 10 ⁻⁴ 5 - 7 10 ⁻⁵ 4 - 0	172+184 171+187	27 - 38	5 min	10 ⁰ 0 - 0 10 ⁻¹ 0 - 0 10 ⁻² 0 - 0 Nts : 0	10 ⁰ 35 - 41 10 ⁻¹ 6 - 4 10 ⁻² 3 - 1 Nts : 9	10 ⁰ >300->300 10 ⁻¹ >300->300 10 ⁻² 165-175 Nts : 1
Log	5,76	5,47	5,72	X = 357,00.10 ⁶ N = 7,25		Nd	< 0,10	< 2,70	5,23
Validation criteria	N - Nc ≤ 2 log	N - NC ≤ 2 log	NC - NT ≤ +/- 0,3	1,5.10 ⁸ ≤ X ≤ 5.10 ⁸ (X = UFC/ML)		ME = Nc - Nd	> 5,66	> 3.06	0,53

Date of the test: Nov/13/2012
 N = Count per ml (1 or 2 plates for N/10⁻⁶)

ESCHERICHIA COLI : Ciel Vert 300B - Batch F0529.12 - Assay 2

VALIDATION				TEST SUSPENSION		TEST			
	Control water	Neutralizer control	Method validation 10 %	N		Contact time	Concentration of product % (V/V)		
	Nc	NC	NT	10 ⁻⁶	10 ⁻⁷		10%	5%	0,1%
X	10 ⁻² 295 - 296 10 ⁻³ 28 - 33 10 ⁻⁴ 2 - 4 10 ⁻⁵ 1 - 1 10 ⁻⁶ 0 - 0 Nts : 1	10 ⁻² > 300 - > 300 10 ⁻³ 70 - 60 10 ⁻⁴ 4 - 8 10 ⁻⁵ 0 - 2	10 ⁻² > 300 - > 300 10 ⁻³ 43 - 50 10 ⁻⁴ 2 - 10 10 ⁻⁵ 0 - 0	146+135 144+119	29 - 31	5 min	10 ⁰ 0 - 0 10 ⁻¹ 0 - 0 10 ⁻² 0 - 0 Nts : 0	10 ⁰ 0 - 0 10 ⁻¹ 0 - 0 10 ⁻² 0 - 0 Nts : 0	10 ⁰ >300->300 10 ⁻¹ >300->300 10 ⁻² >300->300 Nts : 0
Log	5,47	5,81	5,70	X = 272,00.10 ⁶ N = 7,13		Nd	< 0,10	< 0,10	> 5,48
Validation criteria	N - Nc ≤ 2 log	N - NC ≤ 2 log	NC - NT ≤ +/- 0,3	1,5.10 ⁵ ≤ X ≤ 5.10 ⁸ (X = UFC/ML)		ME = Nc - Nd	> 5,37	> 5,37	0

Date of the test: Jan/31/2013
 N = Count per ml (1 or 2 plates for N/10⁻⁶)

VI - CONCLUSION

For the product CIEL VERT 300B (Batch F0529.12), the bactericidal concentration for general purposes determined according to the NF EN 13697 standard (November 2001) (obligatory conditions : 5 min, between 18 to 25°C ± 1°C) under dirty conditions (3 g/l bovine albumin) is 5% (V/V) using *Staphylococcus aureus* CIP 4.83 (ATCC 6538), *Pseudomonas aeruginosa* CIP 103467 (ATCC 15442), *Enterococcus hirae* CIP 5855 (ATCC 10541) and *Escherichia coli* CIP 54127 (ATCC 10536) as test-organisms.

The results hold only for the product under assay.