



Toulouse, October 6th 2009

TEST REPORT N°09-230M

This report replaces and cancels the precedent one (07-09-2009)

STUDY 09-1103

NF EN 1040 - April 2006
BACTERICIDAL ACTIVITY
Obligatory and additional Conditions

Client

CVE
ZI de Pechnaquié
31340 VILLEMUR SUR TARN

Assay Laboratory

FONDEREPHAR
Laboratoire de Microbiologie Industrielle
35 Chemin des Maraîchers
31062 TOULOUSE Cedex 09

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I - IDENTIFICATION OF ASSAY LABORATORY

FONDEREPHAR

Laboratoire de Bactériologie, Virologie et Microbiologie Industrielle
Faculté des Sciences Pharmaceutiques
35 Chemin des Maraîchers
31062 Toulouse cedex 9

II - IDENTIFICATION OF TEST PRODUCT

Product	BLUETOO
Batch.	F41
Date of receipt.	15-06-2009
Internal code.	09-1103
Active Substance.	Not indicated
Promotor.	CVE
Period of testing.	June - July 2009
Storage conditions.	Room temperature

III - TEST

Dilution neutralization method

Neutralizer. Tween 80 (10%), Lecithin (2%), Saponin (2%), Sodium thiosulfate (0.5%), trypticase-soja broth (Batches 626, 627 and 631)

Count per ml : 1

Product diluent. water for injectables preparations

Appearance of the product. Liquid, clear

IV - EXPERIMENTAL CONDITIONS

Product diluent. water for injectables preparations

Obligatory Conditions Test-organism *Pseudomonas aeruginosa* CIP 103467 (ATCC 15442)
Staphylococcus aureus CIP 4.83 (ATCC 6538)

Concentrations of the product. **80% 10%, 1% and 0,1%** (V/V)

Test temperature. $20 \pm 1^{\circ}\text{C}$

Contact time. 5 minutes \pm 10 seconds

Incubation temperature. $36 \pm 1^{\circ}\text{C}$

Additional Conditions Test-organism *Escherichia coli* CIP 54127 (ATCC 10536)

Concentrations of the product. **80% 10%, 1% and 0,1%** (V/V)

Test temperature. $20 \pm 1^{\circ}\text{C}$

Contact time. 5 minutes \pm 10 seconds

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

Concentrations of the product. **80% 10% and 1%** (V/V)

Test temperature. $20 \pm 1^{\circ}\text{C}$

Contact time. 3 minutes \pm 10 seconds

Incubation temperature. $36 \pm 1^{\circ}\text{C}$

No precipitate during the test procedure (test mixtures were homogeneous)

The COFRAC Accreditation only attests the capability of the laboratory for tests NF EN 1040, NF EN 1275, NF EN 1276 and NF EN 1650.

COFRAC is a signatory of the multilateral accord of EA (European co-operation for Accreditation) and of ILAC (International Laboratory Accreditation Cooperation), recognising the equivalence of test reports and analysis.

V - TEST RESULTS

STAPHYLOCOCCUS AUREUS : BLUETOO - Batch F41 - Assay 1

VALIDATION				TEST SUSPENSION		TEST				
	Validation suspension N_{v0}	Neutralizer control B	Method validation 80% C	N et N_0		Concentration of product % (V/V)				
				10^{-6}	10^{-7}		Contact time	80%	10%	1%
Vc1-Vc2	85 - 100	93 - 97	3 min 103-109 5 min 111-102	319->330	28-36	Vc1 - Vc2	3 minutes 5 minutes	0 - 0 0 - 0	0 - 0 0 - 0	4 - 2 0 - 0
\bar{x}	93	95	3 min 106 5 min 107	\bar{x} mp = 319,17.10 ⁶ lg N = 8,50		\bar{x}	3 minutes 5 minutes	< 14 < 14	< 14 < 14	< 14 < 14
				$N_0 = 319,17.10^5$ lg $N_0 = 7,50$		$\bar{N}_a = \bar{x} \times 10$	3 minutes 5 minutes	< 140 < 140	< 140 < 140	< 140 < 140
	$30 < \bar{x}$ of N_{v0} <160	\bar{x} of B $\geq 0,5$ \bar{x} of N_{v0}	\bar{x} of C $\geq 0,5$ \bar{x} of N_{v0}			lg N_a	3 minutes 5 minutes	< 2,15 < 2,15	< 2,15 < 2,15	< 2,15 < 2,15
				$7,17 \leq \lg N_0 \leq 7,70$		lg R	3 minutes 5 minutes	> 5,36 > 5,36	> 5,36 > 5,36	> 5,36 > 5,36

Date of the test: 06-25-2009

Vc = Count per ml (one plate)

\bar{x} = average of Vc1 and Vc2

\bar{x} wm = weighted mean of \bar{x}

R = reduction (lg R = lg N_0 - lg N_a)

FONDEREPHAR

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STAPHYLOCOCCUS AUREUS : BLUETOO - Batch F41 - Assay 2

VALIDATION			TEST SUSPENSION		TEST						
	Validation suspension N_{v0}	Neutralizer control B	Method validation 80% C	N et N_0		Contact time	Concentration of product % (V/V)				
				10^{-6}	10^{-7}		80%	10%	1%	0,1%	
Vc1-Vc2	81 - 82	100 - 77	3 min 71-98 5 min 80-94	271-271	31-31	Vc1 - Vc2	3 minutes 5 minutes	0 - 0 0 - 0	0 - 0 0 - 0	0 - 0 0 - 0	- > 330 - > 330
\bar{x}	82	89	3 min 85 5 min 87	\bar{x} mp = $274,55 \cdot 10^6$ lg N = 8,44		\bar{x}	3 minutes 5 minutes	< 14 < 14	< 14 < 14	< 14 < 14	- > 330
				$N_0 = 274,55 \cdot 10^5$ lg $N_0 = 7,44$		\bar{x}	3 minutes 5 minutes	< 140 < 140	< 140 < 140	< 140 < 140	- > 3300
	$30 < \bar{x}$ of N_{v0} < 160	\bar{x} of B $\geq 0,5$ \bar{x} of N_{v0}	\bar{x} of C $\geq 0,5$ \bar{x} of N_{v0}			lg Na	3 minutes 5 minutes	< 2,15 < 2,15	< 2,15 < 2,15	< 2,15 < 2,15	- > 3,52
				$7,17 \leq \lg N_0 \leq 7,70$		lg R	3 minutes 5 minutes	> 5,29 > 5,29	> 5,29 > 5,29	> 5,29 > 5,29	- < 3,92

Date of the test: 06-30-2009

Vc = Count per ml (one plate)

\bar{x} = average of Vc1 and Vc2

\bar{x} wm = weighted mean of \bar{x}

R = reduction (lg R = lg N_0 - lg Na)

PSEUDOMONAS AERUGINOSA : BLUETOO - Batch F41 - Assay 1

VALIDATION				TEST SUSPENSION		TEST				
	Validation suspension N_{v0}	Neutralizer control B	Method validation 80% C	N et N_0			Concentration of product % (V/V)			
				10^{-6}	10^{-7}		Contact time	80%	10%	1%
Vc1-Vc2	112 - 89	92 - 84	3 min 100-108 5 min 93-92	>330-317	34-37	Vc1 - Vc2	3 minutes 5 minutes	0 - 0 0 - 0	0 - 0 0 - 0	8 - 8 1 - 3
\bar{x}	101	88	3 min 104 5 min 93	$\bar{x}_{mp} = 323,33 \cdot 10^6$ $\lg N = 8,51$		\bar{x}	3 minutes 5 minutes	< 14 < 14	< 14 < 14	< 14 < 14
	$30 < \bar{x} \text{ of } N_{v0} < 160$	$\bar{x} \text{ of } B \geq 0,5$ $\bar{x} \text{ of } N_{v0}$	$\bar{x} \text{ of } C \geq 0,5$ $\bar{x} \text{ of } N_{v0}$	$N_0 = 323,33 \cdot 10^5$ $\lg N_0 = 7,51$		$N_a = \bar{x} \times 10$	3 minutes 5 minutes	< 140 < 140	< 140 < 140	< 140 < 140
				$7,17 \leq \lg N_0 \leq 7,70$		$\lg N_a$	3 minutes 5 minutes	< 2,15 < 2,15	< 2,15 < 2,15	< 2,15 < 2,15
						$\lg R$	3 minutes 5 minutes	> 5,36 > 5,36	> 5,36 > 5,36	> 5,36 > 5,36

Date of the test: 06-25-2009

Vc = Count per ml (one plate)

\bar{x} = average of Vc1 and Vc2

\bar{x}_{wm} = weighted mean of \bar{x}

R = reduction ($\lg R = \lg N_0 - \lg N_a$)

PSEUDOMONAS AERUGINOSA: BLUETOO - Batch F41 - Assay 2

VALIDATION				TEST SUSPENSION		TEST				
	Validation suspension N_{v0}	Neutralizer control B	Method validation 80% C	N et N_0		Contact time	Concentration of product % (V/V)			
				10^{-6}	10^{-7}		80%	10%	1%	0,1%
Vc1-Vc2	59 - 61	89 - 74	3 min 61-68 5 min 63-67	232-242	19-18	Vc1 - Vc2 3 minutes 5 minutes	0 - 0 0 - 0	0 - 0 0 - 0	21 - 20 8 - 9	- > 330 - > 330
\bar{x}	60	82	3 min 65 5 min 65	\bar{x} mp = 232,27.10 ⁶ lg N = 8,37	\bar{x}	3 minutes 5 minutes	< 14 < 14	< 14 < 14	21 < 14	- > 330
	$30 < \bar{x}$ of N_{v0} <160	\bar{x} of B $\geq 0,5$ \bar{x} of N_{v0}	\bar{x} of C $\geq 0,5$ \bar{x} of N_{v0}	$N_0 = 232,27.10^5$ lg $N_0 = 7,37$	$N_a = \bar{x} \times 10$	3 minutes 5 minutes	< 140 < 140	< 140 < 140	210 < 140	- > 3300
				$7,17 \leq \lg N_0 \leq 7,70$	lg N_a	3 minutes 5 minutes	< 2,15 < 2,15	< 2,15 < 2,15	2,32 < 2,15	- > 3,52
					lg R	3 minutes 5 minutes	> 5,22 > 5,22	> 5,22 > 5,22	5,04 > 5,29	- < 3,85

Date of the test: 06-30-2009

Vc = Count per ml (one plate)

\bar{x} = average of Vc1 and Vc2

\bar{x} wm = weighted mean of \bar{x}

R = reduction (lgR = lg N_0 - lg N_a)

ESCHERICHIA COLI : BLUETOO - Batch F41 - Assay 1

VALIDATION				TEST SUSPENSION		TEST				
	Validation suspension N_{v0}	Neutralizer control B	Method validation 80% C	N et N_0			Concentration of product % (V/V)			
				10^{-6}	10^{-7}		Contact time	80%	10%	1%
Vc1-Vc2	47 - 54	73 - 66	3 min 57-63 5 min 79-91	242-225	22-16	Vc1 - Vc2	3 minutes 5 minutes	0 - 0 0 - 0	0 - 0 0 - 0	> 330 - > 330 55 - 42
\bar{x}	51	70	3 min 60 5 min 85	\bar{x} mp = 229,55.10 ⁶ lg N = 8,36		\bar{x}	3 minutes 5 minutes	< 14 < 14	< 14 < 14	> 330 49
				$N_0 = 229,55.10^5$ lg $N_0 = 7,36$		$\bar{N}_a = \bar{x} \times 10$	3 minutes 5 minutes	< 140 < 140	< 140 < 140	> 3300 490
	$30 < \bar{x}$ of N_{v0} < 160	\bar{x} of B $\geq 0,5$ \bar{x} of N_{v0}	\bar{x} of C $\geq 0,5$ \bar{x} of N_{v0}			lg N_a	3 minutes 5 minutes	< 2,15 < 2,15	< 2,15 < 2,15	> 3,52 2,69
				$7,17 \leq \lg N_0 \leq 7,70$		lg R	3 minutes 5 minutes	> 5,21 > 5,21	> 5,21 > 5,21	< 3,84 4,67

Date of the test: 06-25-2009

Vc = Count per ml (one plate)

\bar{x} = average of Vc1 and Vc2

\bar{x} wm = weighted mean of \bar{x}

R = reduction (lg R = lg N_0 - lg N_a)

ESCHERICHIA COLI : BLUETOO - Batch F41 - Assay 2

VALIDATION				TEST SUSPENSION		TEST				
	Validation suspension N_{v0}	Neutralizer control B	Method validation 80% C	N et N_0			Concentration of product % (V/V)			
				10^{-6}	10^{-7}		Contact time	80%	10%	1%
Vc1-Vc2	74 - 57	62 - 71	3 min 85-79 5 min 83-62	225-240	22-28	Vc1 - Vc2	3 minutes 5 minutes	0 - 0 0 - 0	0 - 0 0 - 0	0 - 0 0 - 0
\bar{x}	66	67	3 min 82 5 min 73	\bar{x} mp = 234,09.10 ⁶ lg N = 8,37		\bar{x}	3 minutes 5 minutes	< 14 < 14	< 14 < 14	< 14 < 14
				$N_0 = 234,09.10^5$ lg $N_0 = 7,37$		$\bar{N}_a = \bar{x} \times 10$	3 minutes 5 minutes	< 140 < 140	< 140 < 140	< 140 < 140
	$30 < \bar{x}$ of N_{v0} <160	\bar{x} of B $\geq 0,5$ \bar{x} of N_{v0}	\bar{x} of C $\geq 0,5 \bar{x}$ of N_{v0}	$7,17 \leq \lg N_0 \leq 7,70$		lg N_a	3 minutes 5 minutes	< 2,15 < 2,15	< 2,15 < 2,15	< 2,15 < 2,15
						lg R	3 minutes 5 minutes	> 5,22 > 5,22	> 5,22 > 5,22	> 5,22 > 5,22

Date of the test: 06-30-2009

Vc = Count per ml (one plate)
 \bar{x} = average of Vc1 and Vc2

\bar{x} wm = weighted mean of \bar{x}
R = reduction (lgR = lg N_0 - lg N_a)

ESCHERICHIA COLI : BLUETOO - Batch F41 - Assay 3

VALIDATION				TEST SUSPENSION		TEST					
	Validation suspension N_{v0}	Neutralizer control B	Method validation 80% C	N et N_0		Concentration of product % (V/V)	Contact time	80%	10%	1%	0,1%
				10^{-6}	10^{-7}						
Vc1-Vc2	50 - 62	63 - 67	3 min 70-68 5 min 65-60	208-191	26-30	Vc1 - Vc2	3 minutes 5 minutes	-	-	> 330 - > 330 1 - 5	- > 330 - > 330
\bar{x}	56	65	3 min 69 5 min 63	\bar{x} mp = 206,82.10 ⁶ lg N = 8,32		\bar{x}	3 minutes 5 minutes	-	-	> 330 < 14	- > 330
	$30 < \bar{x}$ of N_{v0} <160	\bar{x} of B $\geq 0,5$ \bar{x} of N_{v0}	\bar{x} of C $\geq 0,5$ \bar{x} of N_{v0}	$N_0 = 206,82.10^5$ lg $N_0 = 7,32$		$N_a = \bar{x} \times 10$	3 minutes 5 minutes	-	-	> 3300 < 140	- < 140
				$7,17 \leq \lg N_0 \leq 7,70$		lg N_a	3 minutes 5 minutes	-	-	> 3,52 < 2,15	- < 2,15
						lg R	3 minutes 5 minutes	-	-	< 3,80 > 5,17	- < 3,80

Date of the test: 07-07-2009

Vc = Count per ml (one plate)

\bar{x} = average of Vc1 and Vc2

\bar{x} wm = weighted mean of \bar{x}

R = reduction (lgR = lg N_0 - lg N_a)

VI - CONCLUSION

For the product BLUETOO - batch F41, the basic bactericidal concentration determined according to the NF EN 1040 standard (obligatory conditions: 5 min, 20°C) is 1% (V/V).

In additional conditions the product BLUETOO - batch F41:

- has bactericidal activity on *Escherichia coli* CIP 54 127 according to the NF EN 1040 standard (obligatory conditions: 5 min, 20°C) at the concentration of 1% (V/V).
- has bactericidal activity on *Pseudomonas aeruginosa* CIP 103467, *Staphylococcus aureus* CIP 4.83 and *Escherichia coli* CIP 54 127, at 20°C, with 3 min contact time, at the concentration of 1% (V/V).