

Toulouse, September 27<sup>th</sup> 2010

## TEST REPORT N°09-230M2

This report replaces and cancels the precedent one (06-10-2009)

### STUDY 09-1103

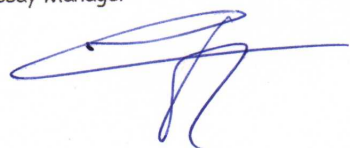
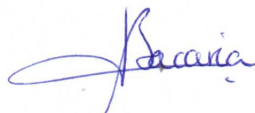
**NF EN 1040 - April 2006**  
**BACTERICIDAL ACTIVITY**  
**Obligatory and additional Conditions**

**Client**

**CVE**  
**ZI de Pechnaugué**  
**31340 VILLEMUR SUR TARN**

**Assay Laboratory**

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

|                                                                                                                                                  |                                                                                                                                                       |                                                                                                                                                              |
|--------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Report drafting<br><br>Sylvie SALVATICO<br>Technician<br><br> | Report control<br><br>Dr Christine ROQUES<br>Assay Manager<br><br> | Report certifying<br><br>Dr Jocelyne BACARIA<br>Quality Manager<br><br> |
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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%), trypcase-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)

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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<br>$N_{v0}$  | Neutralizer control<br>B                           | Method validation<br>80%<br>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<br>5 min 111-102                   | 319->330                                             | 28-36     | Vc1 - Vc2                        | 3 minutes<br>5 minutes | 0 - 0<br>0 - 0   | 0 - 0<br>0 - 0   | 4 - 2<br>0 - 0   |
| $\bar{x}$  | 93                                 | 95                                                 | 3 min 106<br>5 min 107                           | $\bar{x}$ mp = 319,17.10 <sup>6</sup><br>lg N = 8,50 |           | $\bar{x}$                        | 3 minutes<br>5 minutes | < 14<br>< 14     | < 14<br>< 14     | < 14<br>< 14     |
|            |                                    |                                                    |                                                  | $N_0$ = 319,17.10 <sup>5</sup><br>lg $N_0$ = 7,50    |           | $\bar{x} \times 10$<br>Na=       | 3 minutes<br>5 minutes | < 140<br>< 140   | < 140<br>< 140   | < 140<br>< 140   |
|            | $30 < \bar{x}$ of $N_{v0}$<br><160 | $\bar{x}$ of B $\geq 0,5$<br>$\bar{x}$ of $N_{v0}$ | $\bar{x}$ of C<br>$\geq 0,5 \bar{x}$ of $N_{v0}$ | 7,17 $\leq$ lg $N_0 \leq$ 7,70                       |           | lg Na                            | 3 minutes<br>5 minutes | < 2,15<br>< 2,15 | < 2,15<br>< 2,15 | < 2,15<br>< 2,15 |
|            |                                    |                                                    |                                                  |                                                      |           | lg R                             | 3 minutes<br>5 minutes | > 5,36<br>> 5,36 | > 5,36<br>> 5,36 | > 5,36<br>> 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 ( $\lg R = \lg N_0 - \lg Na$ )

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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                                 | 3 min 71-98<br>5 min 80-94                                       | 271-271                                     | 31-31           | Vc1 - Vc2                 | 3 minutes<br>5 minutes | 0 - 0<br>0 - 0                   | 0 - 0<br>0 - 0   | 0 - 0<br>0 - 0   | -<br>> 330 - > 330 |
| $\bar{x}$                      | 82                                      | 3 min 85<br>5 min 87                                             | $\bar{x}_{mp} = 274,55.10^6$<br>lg N = 8,44 |                 | $\bar{x}$                 | 3 minutes<br>5 minutes | < 14<br>< 14                     | < 14<br>< 14     | < 14<br>< 14     | -<br>> 330         |
|                                |                                         |                                                                  | $N_0 = 274,55.10^5$<br>lg $N_0 = 7,44$      |                 | $\bar{x} \times 10$<br>Na | 3 minutes<br>5 minutes | < 140<br>< 140                   | < 140<br>< 140   | < 140<br>< 140   | -<br>> 3300        |
|                                | $30 < \bar{x} \text{ of } N_{v0} < 160$ | $\bar{x} \text{ of } C \geq 0,5$<br>$\bar{x} \text{ of } N_{v0}$ | $7,17 \leq \lg N_0 \leq 7,70$               |                 | lg Na                     | 3 minutes<br>5 minutes | < 2,15<br>< 2,15                 | < 2,15<br>< 2,15 | < 2,15<br>< 2,15 | -<br>> 3,52        |
|                                |                                         |                                                                  |                                             |                 | lg R                      | 3 minutes<br>5 minutes | > 5,29<br>> 5,29                 | > 5,29<br>> 5,29 | > 5,29<br>> 5,29 | -<br>< 3,92        |

Date of the test: 06-30-2009

Vc = Count per ml (oné plate)

$\bar{x}$  = average of Vc1 and Vc2

$\bar{x}_{wm}$  = weighted mean of  $\bar{x}$   
R = reduction ( $\lg R = \lg N_0 - \lg Na$ )

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**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              | 3 min 100-108<br>5 min 93-92 | >330-317                                    | 34-37           | Vc1 - Vc2                 | 3 minutes<br>5 minutes           | 0 - 0<br>0 - 0   | 0 - 0<br>0 - 0   | 8 - 8<br>1 - 3   |
| $\bar{x}$                      | 101                   | 3 min 104<br>5 min 93        | $\bar{x}_{mp} = 323,33.10^6$<br>lg N = 8,51 |                 | $\bar{x}$                 | 3 minutes<br>5 minutes           | < 14<br>< 14     | < 14<br>< 14     | < 14<br>< 14     |
|                                |                       |                              | $N_0 = 323,33.10^5$<br>lg $N_0 = 7,51$      |                 | $N_0 = \bar{x} \times 10$ | 3 minutes<br>5 minutes           | < 140<br>< 140   | < 140<br>< 140   | < 140<br>< 140   |
|                                |                       |                              | $7,17 \leq \lg N_0 \leq 7,70$               |                 | lg Na                     | 3 minutes<br>5 minutes           | < 2,15<br>< 2,15 | < 2,15<br>< 2,15 | < 2,15<br>< 2,15 |
|                                |                       |                              |                                             |                 | lg R                      | 3 minutes<br>5 minutes           | > 5,36<br>> 5,36 | > 5,36<br>> 5,36 | > 5,36<br>> 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 (lgR = lgN<sub>0</sub> - lgNa)

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**PSEUDOMONAS AERUGINOSA: BLUETOO - Batch F41 - Assay 2**

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

Date of the test: 06-30-2009

$V_c$  = 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 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%              |
| Vc1-Vc2    | 47 - 54                                 | 73 - 66                                                          | 3 min 57-63<br>5 min 79-91                                       | 242-225                                              | 22-16     | Vc1 - Vc2                 | 3 minutes<br>5 minutes           | 0 - 0<br>0 - 0   | 0 - 0<br>0 - 0   |
| $\bar{x}$  | 51                                      | 70                                                               | 3 min 60<br>5 min 85                                             | $\bar{x}$ mp = 229,55.10 <sup>6</sup><br>lg N = 8,36 |           | $\bar{x}$                 | 3 minutes<br>5 minutes           | < 14<br>< 14     | < 14<br>< 14     |
|            |                                         |                                                                  |                                                                  | $N_0 = 229,55.10^5$<br>lg $N_0 = 7,36$               |           | $N_0 = \bar{x} \times 10$ | 3 minutes<br>5 minutes           | < 140<br>< 140   | < 140<br>< 140   |
|            | $30 < \bar{x} \text{ of } N_{v0} < 160$ | $\bar{x} \text{ of } B \geq 0,5$<br>$\bar{x} \text{ of } N_{v0}$ | $\bar{x} \text{ of } C$<br>$\geq 0,5 \bar{x} \text{ of } N_{v0}$ | $7,17 \leq \lg N_0 \leq 7,70$                        |           | lg Na                     | 3 minutes<br>5 minutes           | < 2,15<br>< 2,15 | < 2,15<br>< 2,15 |
|            |                                         |                                                                  |                                                                  |                                                      |           | lg R                      | 3 minutes<br>5 minutes           | > 5,21<br>> 5,21 | > 5,21<br>> 5,21 |

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 ( $\lg R = \lg N_0 - \lg Na$ )

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**ESCHERICHIA COLI : BLUETOO - Batch F41 - Assay 2**

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

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Date of the test: 07-07-2009

$x$  = average of  $V_{c1}$  and  $V_{c2}$

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

## 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 against *Pseudomonas aeruginosa* CIP 103467 and *Staphylococcus aureus* CIP 4.83, the product BLUETOO - batch F41 has bactericidal activity at 20°C, with 3 min contact time, at the concentration of 1% (V/V).

On additionnal strain, *Escherichia coli* CIP 54 127 :

- the product BLUETOO - batch F41 has bactericidal activity according to the NF EN 1040 standard (obligatory conditions: 5 min, 20°C) at the concentration of 1% (V/V).
- the product BLUETOO - batch F41 has bactericidal activity, at 20°C, with 3 min contact time, at the concentration of 10% (V/V).