



Akrediteeritud L236

EVS-EN 13727:2012+A1:2013  
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**Quantitative suspension test for the evaluation of bactericidal activity in the medical area (phase 2, step 1)**

TEST REPORT no 244

**1. General information and material**

|                              |                                                              |
|------------------------------|--------------------------------------------------------------|
| 1.1 Client:                  | Medi-Sept Sp. z o.o., Konopnica 159 c, 21-030 Motycz, Poland |
| Date of order:               | 06.11.2015                                                   |
| 1.2 Identification of sample |                                                              |
| Name of the product:         | MEDI-SPRAY                                                   |
| Batch number:                | 151030_50                                                    |
| Manufacturer:                | Medi-Sept Sp. z.o.o.                                         |
| Date of delivery:            | 09.11.2015                                                   |
| Storage conditions:          | room temperature and darkness                                |
| Apperance of the product:    | liquid, clear, without color                                 |
| Recommended diluent:         | product is ready for use                                     |
| Active substance:            | 55-65 % Ethanol and 5-10 % Propan-2-ol                       |

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**1.3 Test conditions**

|                              |                                                                                                                        |
|------------------------------|------------------------------------------------------------------------------------------------------------------------|
| Test period:                 | 11.11.2015 – 13.11.2015                                                                                                |
| Date of test:                | 11.11.2015                                                                                                             |
| Product test concentrations: | 97 %                                                                                                                   |
| Exposure time:               | 15 s., 30 s., 60 s.                                                                                                    |
| Test temperature:            | 19,5 ± 0,5°C                                                                                                           |
| Organic load:                | dirty conditions (bovine albumine 3,0 g/l and sheep erythrocytes 3 ml/l)<br>clean conditions (bovine albumine 0,3 g/l) |
| Neutralizer:                 | Polysorbate 80, 30 g/l; Saponin 30 g/l, Lecithin, 3 g/l                                                                |
| Test organisms:              | Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 15442,<br>Enterococcus hirae ATCC 10541                   |

**2. Methods**

2.1. Test method and its validation: dilution neutralisation

**3. Results**

see annex

**4. Conclusion**

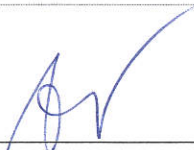
In accordance with EN 13727:2013, product MEDI-SPRAY with concentration 97 % possesses bactericidal activity in suspension test in 30 s. and 60 s. at 20 °C under dirty and clean conditions for referenced strains Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 15442 and Enterococcus hirae ATCC 10541, and in 15 s. under clean conditions for referenced strains Staphylococcus aureus ATCC 6538 and Enterococcus hirae ATCC 10541. The product MEDI-SPRAY demonstrates at least a 5 lg reduction.

Total 11 pages

Annex on 9 pages

Maardu, 13.11.2015

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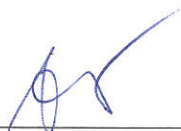


## Annex 1

**VALIDATION AND CONTROLS**

| Test organisms                          | Validation suspension<br>Nv<br>Dilution step<br>-1 |     |                | Validation suspension<br>NvB<br>Dilution step<br>-2 |     |                | Experimental conditions<br>control<br>A<br>Dilution step<br>1 |     |                | Neutralizer control<br>B<br>Dilution step<br>-2 |     |                | Method validation<br>C<br>Dilution step<br>1 |     |                |
|-----------------------------------------|----------------------------------------------------|-----|----------------|-----------------------------------------------------|-----|----------------|---------------------------------------------------------------|-----|----------------|-------------------------------------------------|-----|----------------|----------------------------------------------|-----|----------------|
|                                         | Vc1                                                | Vc2 | X <sup>-</sup> | Vc1                                                 | Vc2 | X <sup>-</sup> | Vc1                                                           | Vc2 | X <sup>-</sup> | Vc1                                             | Vc2 | X <sup>-</sup> | Vc1                                          | Vc2 | X <sup>-</sup> |
| Staphylococcus aureus<br>ATCC 6538      | 75                                                 | 63  | 69             | 44                                                  | 35  | 40             | 88                                                            | 71  | 80             | 50                                              | 39  | 45             | 60                                           | 79  | 70             |
| Pseudomonas<br>aeruginosa ATCC<br>15442 | 60                                                 | 52  | 56             | 35                                                  | 37  | 36             | 53                                                            | 44  | 49             | 40                                              | 34  | 37             | 48                                           | 43  | 46             |
| Enterococcus hirae<br>ATCC 10541        | 35                                                 | 40  | 38             | 68                                                  | 73  | 70             | 30                                                            | 31  | 31             | 50                                              | 54  | 52             | 38                                           | 32  | 35             |

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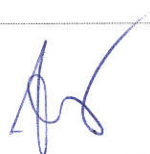



## Annex 2

## TEST SUSPENSION

| Test organisms                    | N  | Vc1 | Vc2 | No                                                             |
|-----------------------------------|----|-----|-----|----------------------------------------------------------------|
| Staphylococcus aureus ATCC 6538   | -7 | 257 | 288 | $N = 2,8 \times 10^9 = \lg 9,45$<br>$No = N / 100 = \lg 7.45$  |
|                                   | -8 | 33  | 35  | $7.17 \leq \lg No \leq 7.70$                                   |
| Pseudomonas aeruginosa ATCC 15442 | -7 | 295 | 274 | $N = 2,83 \times 10^9 = \lg 9,45$<br>$No = N / 100 = \lg 7.45$ |
|                                   | -8 | 25  | 30  | $7.17 \leq \lg No \leq 7.70$                                   |
| Enterococcus hirae ATCC 10541     | -7 | 300 | 300 | $N = 3,01 \times 10^9 = \lg 9,47$<br>$No = N / 10 = \lg 7.47$  |
|                                   | -8 | 38  | 26  | $7.17 \leq \lg No \leq 7.70$                                   |

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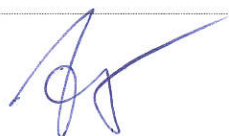


## Annex 3

TEST 1 Staphylococcus aureus  
Clean conditions

| Test organism                   | Dilution step | Vc1 | Vc2 | Na x 10 | lg Na  | lg R | Contact time |
|---------------------------------|---------------|-----|-----|---------|--------|------|--------------|
| Staphylococcus aureus ATCC 6538 | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 15 s.        |
|                                 | -1            | 0   | 0   |         |        |      |              |
|                                 | -2            | 0   | 0   |         |        |      |              |
|                                 | -3            | 0   | 0   |         |        |      |              |
|                                 | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 30 s.        |
|                                 | -1            | 0   | 0   |         |        |      |              |
|                                 | -2            | 0   | 0   |         |        |      |              |
|                                 | -3            | 0   | 0   |         |        |      |              |
|                                 | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 60 s.        |
|                                 | -1            | 0   | 0   |         |        |      |              |
|                                 | -2            | 0   | 0   |         |        |      |              |
|                                 | -3            | 0   | 0   |         |        |      |              |

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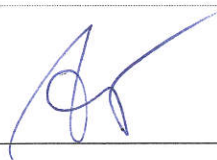



## Annex 4

TEST 2 Staphylococcus aureus  
 Dirty conditions

| Test organism                   | Dilution step | Vc1 | Vc2 | Na x 10 | lg Na  | lg R | Contact time |
|---------------------------------|---------------|-----|-----|---------|--------|------|--------------|
| Staphylococcus aureus ATCC 6538 | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 15 s.        |
|                                 | -1            | 0   | 0   |         |        |      |              |
|                                 | -2            | 0   | 0   |         |        |      |              |
|                                 | -3            | 0   | 0   |         |        |      |              |
|                                 | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 30 s.        |
|                                 | -1            | 0   | 0   |         |        |      |              |
|                                 | -2            | 0   | 0   |         |        |      |              |
|                                 | -3            | 0   | 0   |         |        |      |              |
|                                 | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 60 s.        |
|                                 | -1            | 0   | 0   |         |        |      |              |
|                                 | -2            | 0   | 0   |         |        |      |              |
|                                 | -3            | 0   | 0   |         |        |      |              |

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## Annex 5

TEST 3 *Pseudomonas aeruginosa*  
Clean conditions

| Test organism                            | Dilution step | Vc1 | Vc2 | Na x 10 | lg Na  | lg R | Contact time |
|------------------------------------------|---------------|-----|-----|---------|--------|------|--------------|
| <i>Pseudomonas aeruginosa</i> ATCC 15442 | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 15 s.        |
|                                          | -1            | 0   | 0   |         |        |      |              |
|                                          | -2            | 0   | 0   |         |        |      |              |
|                                          | -3            | 0   | 0   |         |        |      |              |
|                                          | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 30 s.        |
|                                          | -1            | 0   | 0   |         |        |      |              |
|                                          | -2            | 0   | 0   |         |        |      |              |
|                                          | -3            | 0   | 0   |         |        |      |              |
|                                          | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 60 s.        |
|                                          | -1            | 0   | 0   |         |        |      |              |
|                                          | -2            | 0   | 0   |         |        |      |              |
|                                          | -3            | 0   | 0   |         |        |      |              |

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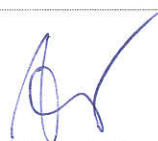



## Annex 6

TEST 4 *Pseudomonas aeruginosa*  
 Dirty conditions

| Test organism                            | Dilution step | Vc1 | Vc2 | Na x 10 | Ig Na  | Ig R | Contact time |
|------------------------------------------|---------------|-----|-----|---------|--------|------|--------------|
| <i>Pseudomonas aeruginosa</i> ATCC 15442 | 1             | 97  | 84  | 910     | 2,96   | 4,49 | 15 s.        |
|                                          | -1            | 6   | 4   |         |        |      |              |
|                                          | -2            | 0   | 0   |         |        |      |              |
|                                          | -3            | 0   | 0   |         |        |      |              |
|                                          | 1             | 28  | 20  | 240     | 2,38   | 5,07 | 30 s.        |
|                                          | -1            | 0   | 0   |         |        |      |              |
|                                          | -2            | 0   | 0   |         |        |      |              |
|                                          | -3            | 0   | 0   |         |        |      |              |
|                                          | 1             | 0   | 0   | < 140   | < 2.15 | >5.3 | 60 s.        |
|                                          | -1            | 0   | 0   |         |        |      |              |
|                                          | -2            | 0   | 0   |         |        |      |              |
|                                          | -3            | 0   | 0   |         |        |      |              |

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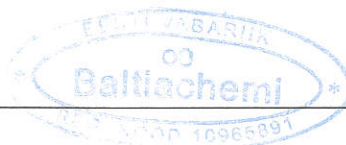



## Annex 7

TEST 5 Enterococcus hirae  
Clean conditions

| Test organism                 | Dilution step | Vc1 | Vc2 | Na x 10 | Ig Na  | Ig R  | Contact time |
|-------------------------------|---------------|-----|-----|---------|--------|-------|--------------|
| Enterococcus hirae ATCC 10541 | 1             | 0   | 0   | < 140   | < 2.15 | >5.32 | 15 s.        |
|                               | -1            | 0   | 0   |         |        |       |              |
|                               | -2            | 0   | 0   |         |        |       |              |
|                               | -3            | 0   | 0   |         |        |       |              |
|                               | 1             | 0   | 0   | < 140   | < 2.15 | >5.32 | 30 s.        |
|                               | -1            | 0   | 0   |         |        |       |              |
|                               | -2            | 0   | 0   |         |        |       |              |
|                               | -3            | 0   | 0   |         |        |       |              |
|                               | 1             | 0   | 0   | < 140   | < 2.15 | >5.32 | 60 s.        |
|                               | -1            | 0   | 0   |         |        |       |              |
|                               | -2            | 0   | 0   |         |        |       |              |
|                               | -3            | 0   | 0   |         |        |       |              |

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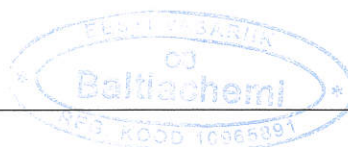



## Annex 8

TEST 6 Enterococcus hirae  
Dirty conditions

| Test organism                 | Dilution step | Vc1 | Vc2 | Na x 10 | lg Na  | lg R  | Contact time |
|-------------------------------|---------------|-----|-----|---------|--------|-------|--------------|
| Enterococcus hirae ATCC 10541 | 1             | 0   | 0   | < 140   | < 2.15 | >5.32 | 15 s.        |
|                               | -1            | 0   | 0   |         |        |       |              |
|                               | -2            | 0   | 0   |         |        |       |              |
|                               | -3            | 0   | 0   |         |        |       |              |
|                               | 1             | 0   | 0   | < 140   | < 2.15 | >5.32 | 30 s.        |
|                               | -1            | 0   | 0   |         |        |       |              |
|                               | -2            | 0   | 0   |         |        |       |              |
|                               | -3            | 0   | 0   |         |        |       |              |
|                               | 1             | 0   | 0   | < 140   | < 2.15 | >5.32 | 60 s.        |
|                               | -1            | 0   | 0   |         |        |       |              |
|                               | -2            | 0   | 0   |         |        |       |              |
|                               | -3            | 0   | 0   |         |        |       |              |

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## Annex 9

$$N = \frac{C}{(n1 + 0.1 n2) \times 10^{-7}}$$

$$Na = c \times 10 / n$$

$$R = \lg No - \lg Na$$

N – is the number of colonies for 1 ml test suspension  
Vc1, Vc2 - is the is number of colonies for 1 ml sample  
n – is the number of Vc-values taken into account  
R – reduction

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