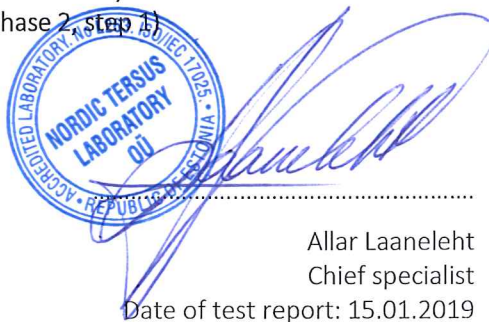


Test report No. sd3919

EVALUATION OF BACTERICIDAL ACTIVITIES OF A DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA (EN 13727)

Name of the product: STERISEPT PLUS  
Batch number: 212310518  
Order number: 18014  
Manufacturer: Chemi-Pharm Ltd  
Client, representative: Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA  
Maris Millner, +372-51-77-090  
Date of delivery: 04.10.2018  
Test material conditions: No specific features, sample in the manufacturers tare  
Storage conditions: In room temperature, dark  
Active substance – conc.: N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine 12.5%; Didecyl-Dimethyl-Ammonium Chloride (DDAC) 12.5%  
Appearance of the product: Transparent liquid  
Test concentration: 0.50% for dirty conditions; 0.25% for clean conditions  
Contact time: 15 min  
Interfering substance: 0.3 g/l bovine albumin = clean conditions; 3.0 g/l bovine albumin + 3ml/l sheep blood erythrocytes = dirty conditions  
Neutralizer: -  
Rinsing liquid: Tryptone 1 g/l + NaCl, 9 g/l  
Test organisms: *Pseudomonas aeruginosa* ATCC 15442  
*Staphylococcus aureus* ATCC 6538  
*Enterococcus hirae* ATCC 10541  
*Staphylococcus aureus* MRSA ATCC 33592  
*Enterococcus faecium* VRE ATCC 700221  
Testing method: EVS-EN 13727:2012+A2:2015  
Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1)  
Testing date: 26.11.2018 – 27.10.2019  
Results: look appendix 1-6

  
Allar Laaneleht  
Chief specialist  
Date of test report: 15.01.2019

Appendix 1

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;  
Membrane filtration method;  
Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l;  
Test organism: *Staphylococcus aureus* ATCC 6538;  
Test temperature: +20° C; Incubation temperature: +37 °C  
Interfering substance: 0.3 g/l bovine albumin = clean conditions; 3.0 g/l bovine albumin + 3ml/l sheepblood erythrocytes = dirty conditions;  
Nordic Tersus Laboratory LLC.;  
Date of test: 26.11.2018  
Responsible person: Allar Laaneleht

Validation and controls

Dirty and clean conditions

| Validation suspension $N_{vo}$                                         |    |                  | Experimental conditions control (A)                                           |    |                  | Filtration control (B)                                                        |    |                  | Method validation (C)                                                         |    |                  |
|------------------------------------------------------------------------|----|------------------|-------------------------------------------------------------------------------|----|------------------|-------------------------------------------------------------------------------|----|------------------|-------------------------------------------------------------------------------|----|------------------|
| $V_{C1}$                                                               | 49 | $\bar{x} = 45.5$ | $V_{C1}$                                                                      | 36 | $\bar{x} = 34.5$ | $V_{C1}$                                                                      | 38 | $\bar{x} = 36.5$ | $V_{C1}$                                                                      | 40 | $\bar{x} = 38.5$ |
| $V_{C2}$                                                               | 42 |                  | $V_{C2}$                                                                      | 33 |                  | $V_{C2}$                                                                      | 35 |                  | $V_{C2}$                                                                      | 37 |                  |
| $30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} A$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} B$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} C$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                  |

Test suspension and test

|                 |           |          |          |                                                                     |
|-----------------|-----------|----------|----------|---------------------------------------------------------------------|
| Testsuspension: | $N$       | $V_{C1}$ | $V_{C2}$ | $\bar{x}_{wm} = 1.60 \times 10^8$ ; $\log N = 8.20$                 |
| $N$ and $N_0$   | $10^{-6}$ | 155      | 163      | $N_0 = N/10$ ; $\log N_0 = 7.20$                                    |
|                 | $10^{-7}$ | 16       | 18       | $7.17 \leq \log N_0 \leq 7.70$ ; yes X; no <input type="checkbox"/> |

Experimental results

| Concentration of the product % | Dilution step | $V_{C1}$ | $V_{C2}$ | $N_a$ ( $=\bar{x} \cdot 10$ ) | $\log N_a$ | $\log R$ | Contact time | Conditions |
|--------------------------------|---------------|----------|----------|-------------------------------|------------|----------|--------------|------------|
| 0.05%                          | -             | >165     | >165     | >1650                         | >3.22      | <3.98    | 15 min       | Clean      |
| 0.25%                          | -             | <14      | <14      | <140                          | <2.15      | >5.05    | 15 min       | Dirty      |
| 0.50%                          | -             | <14      | <14      | <140                          | <2.15      | >5.05    | 15 min       | Clean      |
| 2.50%                          | -             | <14      | <14      | <140                          | <2.15      | >5.05    | 15 min       | Dirty      |

Explanations:

$V_C$  = count per ml (one plate or more)  
 $\bar{x}$  = average of  $V_{C1}$  and  $V_{C2}$  (1. + 2. duplicate)  
 $N$  = cfu/ml microbes in testsuspension  
 $N_0$  = cfu/ml at the start of the contact time (t=0)  
 $N_{vo}$  = cfu/ml in the validation suspension (t=0)  
 $N_a$  = surviving microbes after the test  
 $R$  = reduction factor ( $R = N_0 / N_a$ ;  $\log R = \log N_0 - \log N_a$ )

Appendix 2

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;  
Membrane filtration method;  
Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l;  
Test organism: *Enterococcus hirae* ATCC 10541;  
Test temperature: +20° C; Incubation temperature: +37 °C  
Interfering substance: 0.3 g/l bovine albumin = clean conditions; 3.0 g/l bovine albumin + 3ml/l sheepblood erythrocytes = dirty conditions;  
Nordic Tersus Laboratory LLC.;  
Date of test: 26.11.2018  
Responsible person: Allar Laaneleht

Validation and controls

Dirty and clean conditions

| Validation suspension $N_{vo}$                                         |    |                  | Experimental conditions control (A)                                      |    |                  | Filtration control (B)                                                   |    |                  | Method validation (C)                                                    |    |                |
|------------------------------------------------------------------------|----|------------------|--------------------------------------------------------------------------|----|------------------|--------------------------------------------------------------------------|----|------------------|--------------------------------------------------------------------------|----|----------------|
| $V_{C1}$                                                               | 72 | $\bar{x} = 67.5$ | $V_{C1}$                                                                 | 62 | $\bar{x} = 58.5$ | $V_{C1}$                                                                 | 58 | $\bar{x} = 54.5$ | $V_{C1}$                                                                 | 54 | $\bar{x} = 57$ |
| $V_{C2}$                                                               | 63 |                  | $V_{C2}$                                                                 | 55 |                  | $V_{C2}$                                                                 | 51 |                  | $V_{C2}$                                                                 | 60 |                |
| $30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} A \geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} B \geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} C \geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                |

Test suspension and test

|                                  |           |          |          |                                                                                                                                                                |
|----------------------------------|-----------|----------|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Testsuspension:<br>$N$ and $N_0$ | $N$       | $V_{C1}$ | $V_{C2}$ | $\bar{x}_{wm} = 1.87 \times 10^8$ ; $\log N = 8.27$<br>$N_0 = N/10$ ; $\log N_0 = 7.27$<br>$7.17 \leq \log N_0 \leq 7.70$ ; yes X; no <input type="checkbox"/> |
|                                  | $10^{-6}$ | 179      | 193      |                                                                                                                                                                |
|                                  | $10^{-7}$ | 21       | 18       |                                                                                                                                                                |

Experimental results

| Concentration of the product % | Dilution step | $V_{C1}$ | $V_{C2}$ | $Na$ ( $=\bar{x} \cdot 10$ ) | $\log Na$ | $\log R$ | Contact time | Conditions |
|--------------------------------|---------------|----------|----------|------------------------------|-----------|----------|--------------|------------|
| 0.05%                          | -             | >165     | >165     | >1650                        | >3.22     | <4.05    | 15 min       | Clean      |
| 0.25%                          | -             | <14      | <14      | <140                         | <2.15     | >5.12    | 15 min       | Dirty      |
| 0.50%                          | -             | <14      | <14      | <140                         | <2.15     | >5.12    | 15 min       | Clean      |
| 2.50%                          | -             | <14      | <14      | <140                         | <2.15     | >5.12    | 15 min       | Dirty      |

Explanations:

$V_C$  = count per ml (one plate or more)  
 $\bar{x}$  = average of  $V_{C1}$  and  $V_{C2}$  (1. + 2. duplicate)  
 $N$  = cfu/ml microbes in testsuspension  
 $N_0$  = cfu/ml at the start of the contact time ( $t=0$ )  
 $N_{vo}$  = cfu/ml in the validation suspension ( $t=0$ )  
 $Na$  = surviving microbes after the test  
 $R$  = reduction factor ( $R = N_0 / Na$ ;  $\log R = \log N_0 - \log Na$ )

Appendix 3

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;

Membrane filtration method;

Rinsing liquid: tryptone 1 g/l + NaCl 9 g/l;

Test organism: *Pseudomonas aeruginosa* ATCC 15442

Test temperature: +20° C; Incubation temperature: +37 °C

Interfering substance: 0.3 g/l bovine albumin = clean conditions; 3.0 g/l bovine albumin + 3ml/l sheepblood erythrocytes = dirty conditions;

Nordic Tersus Laboratory LLC.;

Date of test: 26.11.2018

Responsible person: Allar Laaneleht

Validation and controls

Dirty and clean conditions

| Validation suspension $N_{vo}$                                         |    |                  | Experimental conditions control (A)                                           |    |                | Filtration control (B)                                                        |    |                | Method validation (C)                                                         |    |                  |
|------------------------------------------------------------------------|----|------------------|-------------------------------------------------------------------------------|----|----------------|-------------------------------------------------------------------------------|----|----------------|-------------------------------------------------------------------------------|----|------------------|
| $V_{C1}$                                                               | 84 | $\bar{x} = 79.5$ | $V_{C1}$                                                                      | 58 | $\bar{x} = 61$ | $V_{C1}$                                                                      | 67 | $\bar{x} = 69$ | $V_{C1}$                                                                      | 76 | $\bar{x} = 77.5$ |
| $V_{C2}$                                                               | 75 |                  | $V_{C2}$                                                                      | 64 |                | $V_{C2}$                                                                      | 71 |                | $V_{C2}$                                                                      | 79 |                  |
| $30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} A$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                | $\bar{x} B$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                | $\bar{x} C$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                  |

Test suspension and test

|                 |           |          |          |                                                                                                                                                                |
|-----------------|-----------|----------|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Testsuspension: | $N$       | $V_{C1}$ | $V_{C2}$ | $\bar{x}_{wm} = 2.09 \times 10^8$ ; $\log N = 8.32$<br>$N_0 = N/10$ ; $\log N_0 = 7.32$<br>$7.17 \leq \log N_0 \leq 7.70$ ; yes X; no <input type="checkbox"/> |
| $N$ and $N_0$   | $10^{-6}$ | 204      | 215      |                                                                                                                                                                |
|                 | $10^{-7}$ | 22       | 19       |                                                                                                                                                                |

Experimental results

| Concentration of the product % | Dilution step | $V_{C1}$ | $V_{C2}$ | $Na$ ( $=\bar{x} \cdot 10$ ) | $\log Na$ | $\log R$ | Contact time | Conditions |
|--------------------------------|---------------|----------|----------|------------------------------|-----------|----------|--------------|------------|
| 0.05%                          | -             | >165     | >165     | >1650                        | >3.22     | <4.10    | 15 min       | Clean      |
| 0.25%                          | -             | <14      | <14      | <140                         | <2.15     | >5.17    | 15 min       | Dirty      |
| 0.50%                          | -             | <14      | <14      | <140                         | <2.15     | >5.17    | 15 min       | Clean      |
| 2.50%                          | -             | <14      | <14      | <140                         | <2.15     | >5.17    | 15 min       | Dirty      |

Explanations:

$V_C$  = count per ml (one plate or more)

$\bar{x}$  = average of  $V_{C1}$  and  $V_{C2}$  (1. + 2. duplicate)

$N$  = cfu/ml microbes in testsuspension

$N_0$  = cfu/ml at the start of the contact time ( $t=0$ )

$N_{vo}$  = cfu/ml in the validation suspension ( $t=0$ )

$Na$  = surviving microbes after the test

$R$  = reduction factor ( $R = N_0 / Na$ ;  $\log R = \log N_0 - \log Na$ )

Appendix 4

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;

Membrane filtration method;

Rinsing liquid: tryptone 1 g/l + NaCl 9 g/l;

Test organism: *Staphylococcus aureus* MRSA ATCC 33592

Test temperature: +20° C; Incubation temperature: +37 °C

Interfering substance: 0.3 g/l bovine albumin = clean conditions; 3.0 g/l bovine albumin + 3ml/l sheepblood erythrocytes = dirty conditions;

Nordic Tersus Laboratory LLC.;

Date of test: 26.11.2018

Responsible person: Allar Laaneleht

Validation and controls

Dirty and clean conditions

| Validation suspension $N_{vo}$                                         |    |                | Experimental conditions control (A)                                           |    |                | Filtration control (B)                                                        |    |                  | Method validation (C)                                                         |    |                |
|------------------------------------------------------------------------|----|----------------|-------------------------------------------------------------------------------|----|----------------|-------------------------------------------------------------------------------|----|------------------|-------------------------------------------------------------------------------|----|----------------|
| $V_{C1}$                                                               | 60 | $\bar{x} = 57$ | $V_{C1}$                                                                      | 42 | $\bar{x} = 45$ | $V_{C1}$                                                                      | 51 | $\bar{x} = 46.5$ | $V_{C1}$                                                                      | 47 | $\bar{x} = 47$ |
| $V_{C2}$                                                               | 54 |                | $V_{C2}$                                                                      | 48 |                | $V_{C2}$                                                                      | 42 |                  | $V_{C2}$                                                                      | 47 |                |
| $30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/> |    |                | $\bar{x} A$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                | $\bar{x} B$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} C$ is $\geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                |

Test suspension and test

|                 |           |          |          |                                                                     |
|-----------------|-----------|----------|----------|---------------------------------------------------------------------|
| Testsuspension: | $N$       | $V_{C1}$ | $V_{C2}$ | $\bar{x}_{wm} = 1.79 \times 10^8$ ; $\log N = 8.25$                 |
|                 | $10^{-6}$ | 184      | 175      | $N_0 = N/10$ ; $\log N_0 = 7.25$                                    |
| $N$ and $N_0$   | $10^{-7}$ | 16       | 18       | $7.17 \leq \log N_0 \leq 7.70$ ; yes X; no <input type="checkbox"/> |

Experimental results

| Concentration of the product % | Dilution step | $V_{C1}$ | $V_{C2}$ | $Na$ ( $=\bar{x} \times 10$ ) | $\log Na$ | $\log R$ | Contact time | Conditions |
|--------------------------------|---------------|----------|----------|-------------------------------|-----------|----------|--------------|------------|
| 0.05%                          | -             | >165     | >165     | >1650                         | >3.22     | <4.03    | 15 min       | Clean      |
| 0.25%                          | -             | <14      | <14      | <140                          | <2.15     | >5.10    | 15 min       | Dirty      |
| 0.50%                          | -             | <14      | <14      | <140                          | <2.15     | >5.10    | 15 min       | Clean      |
| 2.50%                          | -             | <14      | <14      | <140                          | <2.15     | >5.10    | 15 min       | Dirty      |

Explanations:

$V_C$  = count per ml (one plate or more)

$\bar{x}$  = average of  $V_{C1}$  and  $V_{C2}$  (1. + 2. duplicate)

$N$  = cfu/ml microbes in testsuspension

$N_0$  = cfu/ml at the start of the contact time ( $t=0$ )

$N_{vo}$  = cfu/ml in the validation suspension ( $t=0$ )

$Na$  = surviving microbes after the test

$R$  = reduction factor ( $R = N_0 / Na$ ;  $\log R = \log N_0 - \log Na$ )

Appendix 5

**TEST RESULTS (bactericidal suspension test)**

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;

Membrane filtration method;

Rinsing liquid: tryptone 1 g/l + NaCl 9 g/l;

Test organism: *Enterococcus faecium* VRE ATCC 700221

Test temperature: +20° C; Incubation temperature: +37 °C

Interfering substance: 0.3 g/l bovine albumin = clean conditions; 3.0 g/l bovine albumin + 3ml/l sheepblood erythrocytes = dirty conditions;

Nordic Tersus Laboratory LLC.;

Date of test: 26.11.2018

Responsible person: Allar Laaneleht

Validation and controls

Dirty and clean conditions

| Validation suspension $N_{vo}$                                         |    |                  | Experimental conditions control (A)                                      |    |                | Filtration control (B)                                                   |    |                  | Method validation (C)                                                    |    |                |
|------------------------------------------------------------------------|----|------------------|--------------------------------------------------------------------------|----|----------------|--------------------------------------------------------------------------|----|------------------|--------------------------------------------------------------------------|----|----------------|
| $V_{C1}$                                                               | 58 | $\bar{x} = 54.5$ | $V_{C1}$                                                                 | 40 | $\bar{x} = 40$ | $V_{C1}$                                                                 | 36 | $\bar{x} = 37.5$ | $V_{C1}$                                                                 | 43 | $\bar{x} = 45$ |
| $V_{C2}$                                                               | 51 |                  | $V_{C2}$                                                                 | 40 |                | $V_{C2}$                                                                 | 39 |                  | $V_{C2}$                                                                 | 47 |                |
| $30 \leq \bar{x} N_{vo} \leq 160$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} A \geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                | $\bar{x} B \geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                  | $\bar{x} C \geq 0.5 \bar{x} N_{vo}$ ? yes X; no <input type="checkbox"/> |    |                |

Test suspension and test

|                 |           |          |          |                                                                                                                                                                |
|-----------------|-----------|----------|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Testsuspension: | $N$       | $V_{C1}$ | $V_{C2}$ | $\bar{x}_{wm} = 1.75 \times 10^8$ ; $\log N = 8.24$<br>$N_0 = N/10$ ; $\log N_0 = 7.24$<br>$7.17 \leq \log N_0 \leq 7.70$ ; yes X; no <input type="checkbox"/> |
|                 | $10^{-6}$ | 167      | 181      |                                                                                                                                                                |
| $N$ and $N_0$   | $10^{-7}$ | 17       | 20       |                                                                                                                                                                |

Experimental results

| Concentration of the product % | Dilution step | $V_{C1}$ | $V_{C2}$ | $Na$ ( $=\bar{x} \cdot 10$ ) | $\log Na$ | $\log R$ | Contact time | Conditions |
|--------------------------------|---------------|----------|----------|------------------------------|-----------|----------|--------------|------------|
| 0.05%                          | -             | >165     | >165     | >1650                        | >3.22     | <4.02    | 15 min       | Clean      |
| 0.25%                          | -             | <14      | <14      | <140                         | <2.15     | >5.09    | 15 min       | Dirty      |
| 0.50%                          | -             | <14      | <14      | <140                         | <2.15     | >5.09    | 15 min       | Clean      |
| 2.50%                          | -             | <14      | <14      | <140                         | <2.15     | >5.09    | 15 min       | Dirty      |

Explanations:

$V_C$  = count per ml (one plate or more)

$\bar{x}$  = average of  $V_{C1}$  and  $V_{C2}$  (1. + 2. duplicate)

$N$  = cfu/ml microbes in testsuspension

$N_0$  = cfu/ml at the start of the contact time ( $t=0$ )

$N_{vo}$  = cfu/ml in the validation suspension ( $t=0$ )

$Na$  = surviving microbes after the test

$R$  = reduction factor ( $R = N_0 / Na$ ;  $\log R = \log N_0 - \log Na$ )

Appendix 6

Interpretation:

The product STERISEPT PLUS (batch no. 212310518) was tested according to the test method EVS-EN 13727:2012+A2:2015. The test was performed at  $20\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ , under clean dirty conditions with the contact time of 15 min. The membrane filtration method was used for testing the product's effectiveness against the reference strains: *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecium* VRE ATCC 700221 and *Staphylococcus aureus* MRSA ATCC 33592. Under clean and dirty conditions the tested product was effective against all the reference strains within 15 min.

Conclusion:

The surviving count of bacterial reference strains showed at least 5lg reduction meaning that under dirty conditions the 0.50% solution of product STERISEPT PLUS has a bactericidal effect within 15 min and under clean conditions the 0.25% solution of the product has a bactericidal effect within 15 min.



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Allar Laaneleht  
Chief Specialist

15.01.2019