

Return address: P.O. Box 360, 3700 AJ Zeist. The Netherlands

Coolhands BV. T.a.v. P. Leering Professor van Vlotenweg 3 2061 EB Bloemendaal

Subject Screening test

Dear Mr. Leering,

Please find enclosed the results of the disinfection screening tests TNO performed with your product on two bacteria, Staphylococcus aureus ATCC 6538 and Pseudomonas aeruginosa ATCC 15442. The product was received from Coolhands BV on 12th of April 2016. The product was tested in three concentrations (0.5%, 1.5% and 3%) and during three different contact times (1 min., 5 min. and 10 min).

The screening test was performed applying the exposure condition described in NEN-EN 1276 "Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)" without the validation tests. The requirements for a disinfectant is showing a reduction > 5 Log units for the bacteria in the test. The results of the screening test are shown in Table 1.

From the results we conclude:

- 1. The product shows bactericidal activity on Staphylococcus aureus at a concentration of 0.5%, 1.5% and 3% (v/v) in 1 minute exposure time in distilled water at 20° C.
- 2. The product shows bactericidal activity on Pseudomonas aeruginosa at a concentration of 1.5% (v/v) and 3% (v/v) in distilled water in 1 minute exposure time at 20°C and in 5 minutes exposure time at a concentration of 0.5% (v/v) in distilled water at 20°C.

The test results are valid for this test subject only.

Earth, Life and Social Sciences Utrechtseweg 48 3704 HE Zeist P.O. Box 360 3700 AJ Zeist

www.tno.nl

The Netherlands

T +31 88 866 60 00 F +31 88 866 87 28 infodesk@tno.nl

Date 21 April 2016

Our reference MSB-2016-0100296157 HEM

E-mail margreet.heerikhuisen@tno.nl

Direct dialling +31 88 866 5146

Direct fax +31 30 694 41 08

Project number 060.20946/01.39.01

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Our General Terms and Conditions are also available on our website www.tno.nl.

A copy will be sent upon request.

Trade register number 27376655.



Since the product has achieved the target minimum reduction of 5 log units, this is encouraging for further work which is necessary toward registration of the product as a disinfectant for specific applications.

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Yours faithfully

M. Heerikhuisen

Project manager Microbiology



Table 1. Test results (bactericidal suspension test)

Test organism: Staphylococcus aureus ATCC 6538 Incubation temperature: 37°C

N	Vc1	Vc1	$Xg(wm) = 5,7x10^8$
10 ⁻⁶	>300	>300	logN = 8.76 No=N/10
10 ⁻⁷	65	49	logNo = 7.76 7,17 ≤ logNo ≤ 7,70? yes □ no
	10 ⁻⁶	10 ⁻⁶ >300	10 ⁻⁶ >300 >300

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Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14				
10 ⁻¹			-11	1 15	6.61	1
10-2						
10-3			1 14	1.13	0.01	1
	10 ⁰ 10 ⁻¹ 10 ⁻²	10° <14 10°¹ 10°²	10° <14 <14 10°¹ 10°²	Step (Xg(wm)) 10° <14 <14 10° 10°	10° <14 <14 10°	step (Xg(wm)) (Ig No - Ig Na) 10° <14 <14 10° 10° <14 115 6.61

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14				
10 ⁻¹						
10 ⁻²			<14	1.15	6.61	5
10-3			1 14	1.13	0.01	3

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14				
10 ⁻¹						
10 ⁻²			<14	1.15	6.61	10
10 ⁻³			14	1.15	0.01	10

Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
1.50/	10°	<14	<14				
	10-1			<14	1.15	6.61	1
	10 ⁻²						
1,5%	10-3			1 14	1.15	0.01	1
				1			
				1			

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14				
10-1			1			
10-2			<14	1.15	6.61	5
10 ⁻³			14	1.13	0.01	,

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14				
10-1						
10-2			<14	1.15	6.61	10
10 ⁻³			14	1.13	0.01	10

Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
	10°	<14	<14				
	10-1					6.64	
	10 ⁻²						
3%	10-3			<14	1.15	6.61	1
				1			
				1			

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10 ⁰	<14	<14				
10 ⁻²			<14	1.15	6.61	5

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10-2	10°	<14	<14				
10 ⁻²	10-1				4.45	6.61	
10 ⁻³	10-2						
	10-3			<14	1.15	6.61	10
				1			

Legend

N = number of cfu/ml of the bacterial test suspension

 N_0 = number of cfu/ml in the test suspension at the begin of the exposure time

R = reduction of viability

Vc = viable count

Na = number of cfu/ml in the test mixture at the end of the exposure time



Table 1. Test results (bactericidal suspension test)

Test organism: Pseudomonas aeruginosa ATCC 15442

Incubation temperature: 37°C

Test suspension Vc1 Vc1 $Xg(wm) = 7.5x10^8$ (N and No): log N = 8.8710⁻⁶ >300 >300 No=N/10 10-7 69 81 log**No** = 7.87 $7,17 \le \log No \le 7,70?$ yes \Box no V Date 21 April 2016

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Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)	s
	10°	>330	>330					
	10-1	169	168					
0,5%	10-2	17	18	169	3.23	4.64	1	
0,070	10 ⁻³			103	3.23	4.04		F
								-

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	110	116				
10 ⁻¹	14	12	1			
10-2			115	2.06	5.81	5
10 ⁻³			1	2.00	0.01	

10 ⁰ 35 39 10 ⁻¹	
10-2 37 1.57 6.3	10
10-3	10

Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
1,5%	10°	<14	<14	<14	1.15	6.72	1
	10-1						
	10-2						
	10 ⁻³						

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14				
10-1			1			
10-2				4 45	6 70	_
10 ⁻³			<14	1.15	6.72	5

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14				
10-1			1			
10-2					6 73	4.0
10 ⁻³			<14	1.15	6.72	10
			1			
			1			

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14		4.45		
10-1						
10-2						
10 ⁻³			<14	1.15	6.72	1
			1			
	10 ⁰ 10 ⁻¹ 10 ⁻²	10 ⁰ <14 10 ⁻¹ 10 ⁻²	10° <14 <14 10°¹ 10°²	10° <14 <14 10°¹ 10°²	10° <14 <14 10°¹ 10°²	(Xg(wm)) (Ig No - Ig Na) 10° <14 <14 10° 115 6.73

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14				
10-1						
10 ⁻²			<14	1.15	6.72	5
10 ⁻³			<14	1.15	6.72	5

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10°	<14	<14				
10-1			1			
10 ⁻²			1			
10 ⁻³			<14	1.15	6.72	10

Legend

N = number of cfu/ml of the bacterial test suspension

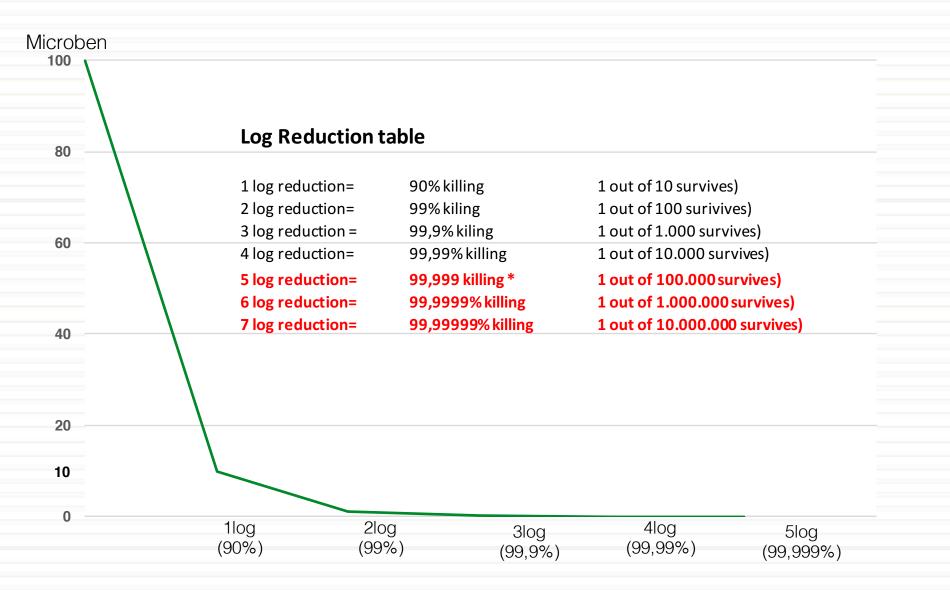
 N_0 = number of cfu/ml in the test suspension at the begin of the exposure time

R = reduction of viability

Vc = viable count

Na = number of cfu/ml in the test mixture at the end of the exposure time

Log Reduction in the disinfection sector



^{* 5} log reduction- minimum requirement hospitals



Screening test performed by: The innovation for life

Result on bacteria Staphylococcus aureus

Concentration PA	Contact time & Log Reduction						
	1 min	5 min	10 min				
0,5%	6,61	6,61	6,61				
1,5%	6,61	6,61	6,61				
3%	6,61	6,61	6,61				



S. <u>Aureus is a common cause of</u>
<u>skin infections such as</u>
<u>abscesses, respiratory infections</u>
<u>such as sinusitis, and food</u>
<u>poisoning.</u>

Result on bacteria Pseudomonas aeruginosa

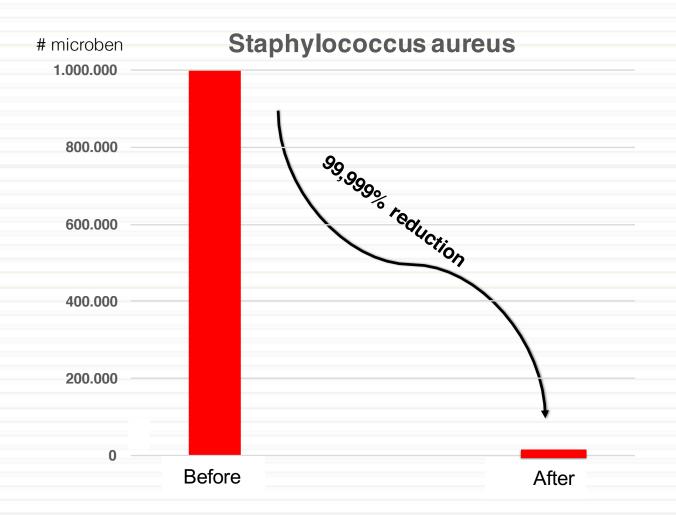
Concentration PA	Contact time & Log Reduction						
	1 min	5 min	10 min				
0,5%	4,64	5,81	6,3				
1,5%	6,72	6,72	6,72				
3%	6,72	6,72	6,72				



, P. aeruginosatypically infects the airway, urinary tract, burns, and wounds, and also causes other blood infections.[



Coolhands wipe: Test bacteriostatic performance





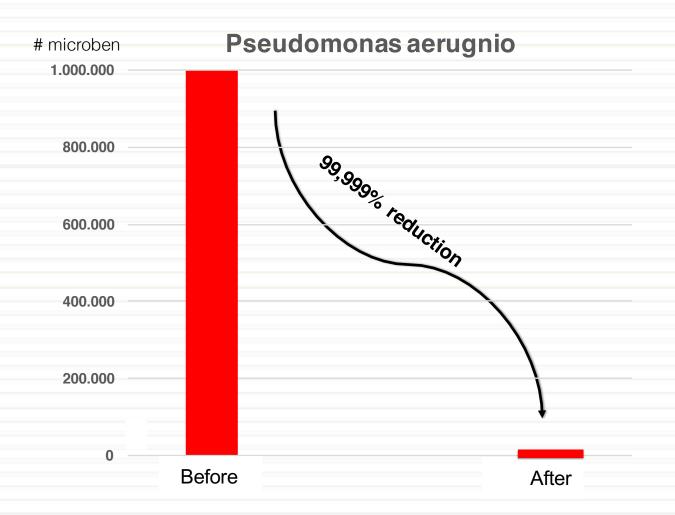


S. <u>Aureus is a common</u>
<u>cause of skin infections such</u>
<u>as abscesses, respiratory</u>
<u>infections such as sinusitis,</u>
<u>and food poisoning.</u>

TNO test executed April 2016, 0,5% Path Away and contact time 5 minutes. Various concentrations and contact times were tested.



Coolhands wipe: Test bacteriostatic performance







, P. aeruginosatypically infects the airway, urinary tract, burns, and wounds, and also causes other blood infections.

TNO test executed April 2016, 0,5% Path Away and contact time 5 minutes. Various concentrations and contact times were tested.

