Henkel AG & Co. KGaA

Microbiology

Test report

21-06176-2

on the
bactericidal efficacy

of

Active Anolyte

according to the principles of DIN EN 13727:
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);
German version EN 13727:2012+A2:2015

Customer:	Mr. Andreas Thul,
	Elements of Water GmbH
Author:	Roland Breves
Date:	September 29, 2021

H e n k e l AG & Co. KGaA
Corporate Scientific Services / Microbiology
D-40191 Duesseldorf

Tel.: +49-211-797 9630
Fax: +49-211-798 2245
e-mail: roland.breves@henkel.com

1. Test laboratory

Henkel AG & Co. KGaA
Corporate Scientific Services / Microbiology
D-40191 Düsseldorf

2. Identity of the test substance

2.1.	Product name	**Active Anolyte**
2.2	Batch	lot no AA44286-01
2.3	Manufacturing date	unknown
2.4	Manufacturer	Elements of Water GmbH
2.5	Date of sample entry	April 09, 2021
2.6	Expiry date	October 2021
2.6	Active substance(s)	Active chlorine released from hypochlorous acid
2.7	Storage conditions in the laboratory	room temperature
2.8	Appearance	clear, colourless liquid
2.9	Physical parameters	(before/after test)
2.9.1	Active chlorine:	500 ppm / 440 ppm
2.9.2	pH:	6.98 / 4.87
2.9.3	Redox potential:	924 mV / 1070 mV

3. Test method and neutralization

3.1 Suspension test according to the principles of DIN EN 13727:

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);

German version EN 13727:2012+A2:2015

Scope of application: Efficacy testing of disinfectants in chemical products including industrial, domestic and institutional areas, food, veterinary medicine and hospital hygiene (with the exception: no testing and statements of conformity of medical devices)

Test parameters have been adjusted to the requirements of VAH 9 and 14.1 (Surface disinfection without mechanical action – simulated-use test in: Requirements and methods for the VAH certification of chemical disinfection processes (2015-04)), as follows:

Three contact times at 80% have been applied instead of 3 concentrations per contact time as prescribed in DIN EN 13727.

3.2 Neutralization by: dilution-neutralization

Neutralizer solution based on:

3% Tween 80, 0.3% Lecithin, 0.1% Histidin, 0.5% Sodium thiosulphate

4. Experimental conditions

4.1	Date of test:	June 28 – July 05, 2021
4.2	Diluent:	distilled water
4.3	Test concentrations:	80%
4.4	Appearance in the test:	colourless liquid, without precipitation
4.5	Test organisms:	*Enterococcus hirae* DSM 3320 (=ATCC 10541)
		Pseudomonas aeruginosa DSM 939 (=ATCC 15442)
		Staphylococcus aureus DSM 799 (=ATCC 6538)
4.6	Contact time(s):	30 sec – 60 sec – 5 min
4.7	Test temperature:	20° C
4.8	Interfering substance:	0.3g/l BSA
4.9	Incubation temperature:	36° C

5. Results

An overview of the achieved reduction factors is given in the table below. Detailed test results are illustrated in the appendix.

The non-interference of the chosen experimental conditions, the absence of toxicity of the selected neutralizer solution as well as the successful validation of the dilution –neutralization method was proven. Thus, the results can be regarded as sufficiently valid.

DIN EN 13727	**Active Anolyte 20°C / low soil (0.3g/l BSA)**			
Test organism ▼	**Reduction factors (RF)**			
	Time ▶ Conc. ▼	**30 sec**	**60 sec**	**5 min**
Staphylococcus aureus DSM 799 (=ATCC 6538)	**80%**	**>5.48**	**>5.48**	**>5.48**
Enterococcus hirae DSM 3320 (=ATCC 10541)	**80%**	**>5.38**	**>5.38**	**>5.38**
Pseudomonas aeruginosa DSM 939 (=ATCC 15442)	**80%**	**>5.47**	**>5.47**	**>5.47**

6. Conclusion

In the standard EN 13727 the minimum requirements for efficacy for a disinfectant are defined as the capability to reduce the viable counts of the test organisms by a factor of >5lg within the defined contact time at the relevant test temperature under the influence of the chosen level of interfering substances. The same level of efficacy is required by VAH 9 and 14.1 for products intended for surface disinfection without mechanical action.

For the product **Active Anolyte** a **sufficiently bactericidal efficacy** against all prescribed test organisms could be achieved at 20° and simulated low soil conditions within 30 sec at undiluted (=80%) product concentration.

Duesseldorf, September 29, 2021

Dr. Roland Breves
Head of Microbiology

Julia deBache-Boy
Head of lab Product Efficacy

Sampling was performed by the customer (if not described otherwise). All results refer only to the samples provided by the customer. A copy of the original signed version of this report and the raw data are filed at Corporate Scientific Services / Microbiology for a storage period of 10 years. The report may only be distributed in complete form without any changes.

Appendix: Detailed test results

Appendix: Test results, validation and controls – bactericidal efficacy EN 13727

DIN EN 13727:2012+A2:2015	LIMS 21-06176	T = 20°	Soil: low (0.03% BSA)	Active Anolyte				
Test organism	Validation and controls				Product tests (5.5.2.2): test conditions : concentration [%] and contact time [sec/min]			
	Validation suspension Nv (N_{VB}) (5.4.1.5)	Validation of the selected experimental conditions A (5.5.2.3)	Verification of the absence of toxicity of the neutralizer B (5.5.2.4)	Validation of the dilution-neutralization method C (5.5.2.5)	Test suspension N (5.4.1.4.)		30 sec	60 sec	5 min
Staphylococcus aureus DSM 799 (=ATCC 6538)	V_c: 138/123 N_{V0}: $1.31x10^2$ [x]yes [ ]no V_c: 112/130 N_{VB}: $1.21x10^5$ [x]yes [ ]no	**5 min** V_c: 125/129 A: $1.27x10^2$ [x]yes [ ]no	V_c: 147/129 B: $1.38x10^2$ [x]yes [ ]no	**30 sec / 80%:** V_c: 147/150 C: $1.49x10^2$ **60 sec / 80%:** V_c: 134/145 C: $1.40x10^2$ **5 min / 80%:** V_c: 133/141 C: $1.37x10^2$ [x]yes [ ]no			**80 %**	**80 %**	**80 %**
					10^{-6} : >330/>330	Vc0:	0 / 0	0 / 0	0 / 0
					10^{-7} : 48 / 37	Vc-1	0 / 0	0 / 0	0 / 0
					N: $4.25x10^8$	Vc-2:	0 / 0	0 / 0	0 / 0
					lg N: 8.63	Na:	$<1.4x10^2$	$<1.4x10^2$	$<1.4x10^2$
					lg N_0: 7.63	lg Na	<2.15	<2.15	<2.15
					[x]yes [ ]no	R:	**>5.48**	**>5.48**	**>5.48**
Enterococcus hirae DSM 3320 (=ATCC 10541)	V_c: 130/126 N_{V0}: $1.28x10^2$ [x]yes [ ]no V_c: 156/139 N_{VB}: $1.48x10^5$ [x]yes [ ]no	**5 min** V_c: 120/104 A: $1.12x10^2$ [x]yes [ ]no	V_c: 130/116 B: $1.81x10^2$ [x]yes [ ]no	**30 sec / 80%:** V_c: 135/130 C: $1.33x10^2$ **60 sec / 80%:** V_c: 156/160 C: $1.58x10^2$ **5 min / 80%:** V_c: 154/143 C: $1.49x10^2$ [x]yes [ ]no			**80 %**	**80 %**	**80 %**
					10^{-6} : >330/>330	Vc0:	0 / 0	0 / 0	0 / 0
					10^{-7} : 33/34	Vc-1	0 / 0	0 / 0	0 / 0
					N: $3.35x10^8$	Vc-2:	0 / 0	0 / 0	0 / 0
					lg N: 8.53	Na:	$<1.4x10^2$	$<1.4x10^2$	$<1.4x10^2$
					lg N_0: 7.53	lg Na	<2.15	<2.15	<2.15
					[x]yes [ ]no	R:	**>5.38**	**>5.38**	**>5.38**
Pseudomonas aeruginosa DSM 939 (=ATCC 15442)	V_c: 126/106 N_{V0}: $1.16x10^2$ [x]yes [ ]no V_c: 111/120 N_{VB}: $1.16x10^5$ [x]yes [ ]no	**5 min** V_c: 70/ 61 A: $6.55x10^1$ [x]yes [ ]no	V_c: 101/107 B: $1.04x10^2$ [x]yes [ ]no	**30 sec / 80%:** V_c: 78/ 93 C: $8.55x10^1$ **60 sec / 80%:** V_c: 95/127 C: $1.11x10^2$ **5 min / 80%:** V_c: 102/128 C: $1.15x10^2$ [x]yes [ ]no			**80 %**	**80 %**	**80 %**
					10^{-6} : >330/>330	Vc0:	0 / 0	0 / 0	0 / 0
					10^{-7} : 45/39	Vc-1	0 / 0	0 / 0	0 / 0
					N: $4.20x10^8$	Vc-2:	0 / 0	0 / 0	0 / 0
					lg N: 8.62	Na:	$<1.4x10^2$	$<1.4x10^2$	$<1.4x10^2$
					lg N_0: 7.62	lg Na	<2.15	<2.15	<2.15
					[x]yes [ ]no	R:	**>5.47**	**>5.47**	**>5.47**

Legend:

V_c:	Colonies counted
Na:	Cell counts in the test assays (after the test)
N:	Cell counts in the test suspension
N_0:	Cell counts in the test assays (before the test)
Nv:	Cell counts in the validation suspension
Nv_0:	Cell counts in the validation test
Nv_B:	Cell counts in the validation suspension B
A:	Cell counts in the experimental conditions control
B:	Cell counts in the neutralizer control (absence of toxicity)
C:	Cell counts in the validation of the neutralization method
R:	Reduction factor

Criteria of validity:

Lg N:	8.17 – 8.70
lg N_0:	7.17 – 7.70
N_{v0} :	30 -160
Nv_B :	$3{,}0 \times 10^4$ - 1.6×10^5
A, C:	each $\geq 0.5\ N_{v0}$
B :	$\geq 0.0005\ Nv_B$