

HYGIENE NORD GMBH  
C/O BIOTECHNIKUM  
WALTHER-RATHENAU-STRASSE 49 A  
17489 GREIFSWALD  
DEUTSCHLAND - GERMANY



HYGIENE NORD GMBH, C/O BIOTECHNIKUM, W.-RATHENAU-STR. 49 A, D-17489 GREIFSWALD

**Geneon Europe ehf**  
**Bæjarhrauni 10**  
**220 Hafnafjordur**  
**Iceland**

CUSTOMER NUMBER  
824

DATE  
March 30, 2015

**REPORT A 15018**  
**SANITIZER/CLEANER (TRIO+)**

### Purpose

The bactericidal activity of the ECA solution **Sanitizer/Cleaner**, prepared by the user using the **Trio+** equipment using blended-stream electro-chemical activation technology (GeneonEurope ehf., Hafnafjord, Iceland) should be evaluated in accordance with the **EN 1276 (2009)**.

Order number: A 15046

Sanitizer/Cleaner (Trio+)

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GfGZ DER GESELLSCHAFT  
GREIFSWALD

HANDELSREGISTER  
AG STRALSUND HRB 5184  
USt-ID (VAT):  
DE21530553

## Test description

Manufacturer:	GeneonEurope ehf., Hafnarfjord, Iceland		
Product name (Solution):	Sanitizer/Cleaner		
Equipment name:	Trio+; 1.2 l jug + base station		
Sample number:	P 150432 (produced solution)		
Batch number:	not provided / not applicable		
Storage conditions:	Room temperature		
Product dilution:	water of standardized hardness		
Date of order:	February 11, 2015		
Date of delivery:	February 13, 2015		
Test time:	March 27, 2015– March 28, 2015		
Basis:	EN 1276 (2009) 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)		
Test organisms:	<i>Escherichia coli</i> K12	ATCC 10536	
	<i>Pseudomonas aeruginosa</i>	ATCC 15442	
	<i>Enterococcus hirae</i>	ATCC 10541	
	<i>Staphylococcus aureus</i>	ATCC 6538	
Test solution:	97 %, 80 %, 50 %, 10 %		
Odor:	fresh (chlorine)		
Appearance (solution):	clear, colourless liquid		
Composition per 100 ml:	not provided (mixture of free oxidizing agents)		
	Redox (DIN 38404-C 6) *	Free Chlorine (DIN EN ISO 7393-2) *	
	P 150432: +950 mV	100 %: 180 mg / l	
	*1 Determined by Industrie- und Umweltlaboratorium Vorpommern GmbH, Am Koppelberg 20,17489 Greifswald		
pH-value:	100 %: 8.43	50 %: 8.31	10 %: 7.85
	WSH: 7.20		
Neutralizer:	3 % Tween 80 + 0.3 % Lecithin + 0.1 % Histidine + 0.5 % Na-Thiosulfate (Neutraliser III)		
Contact time:	5 min		
Interfering substance:	0.03 % albumin (clean conditions)		
Test temperature:	20 ± 1 °C		
Incubation temperature:	36 ± 1 °C		

## Test Method

Testing is based on the European Standard EN 1276 (2009). Validation and control procedures are therefore carried out in accordance with that standard.

The test product solution **Sanitizer/Cleaner** was produced in the lab about 10 min prior to the test using the **Trio+** equipment (1.2 l jug + base station). To that end, and following the manufacturer's recommendations, 3 Doses of the red marked **Sanitizer/Cleaner** salt were added to 1.2 litres of water of standardized hardness (WSH). The test product solution was generated by starting the **electrochemical activation 2 times** (2 cycles, each with 5 min running time) on the same sample, with 1 min gaps between the consecutive activations.

For the test, a sample of the product **Sanitizer/Cleaner** (diluted with hard water if necessary) is added to a suspension of test organisms in a solution of the interfering substance. The mixture is maintained at  $20 \pm 1$  °C for the required contact times. At the end of the contact time, an aliquot of 1 ml is taken; the microbiocidal activity in this portion is immediately neutralized. Two 1 ml samples (and, if applicable: per dilution step) of this suspension are spread on at least 2 plates each or on 1 plate each using the pour-plate-technique. The number of surviving test organisms in the test mixture is calculated for each sample and the reduction is determined with respect to the corresponding test suspension  $N_0$ .

The experimental conditions (control A), the non-toxicity of the neutralizer (control B) and the dilution-neutralization method (control C) are validated. The test is performed using *Staphylococcus aureus*, *Enterococcus hirae*, *Pseudomonas aeruginosa* and *Escherichia coli*. Results are presented in tables 1 – 4.

## Results

In accordance with the EN 1276 (2009), the batch of **Sanitizer/Cleaner** prepared for this test according to the manufacture's recommendation [3 doses salt / 1.2 l WSH; 2 activations (resulting in a chlorine concentration of 180 mg/l)] possesses **bactericidal** activity ( $\log_{10}$  RF  $\geq 5$ ) at  $20 \pm 1$  °C under clean conditions (0.03 % albumin) for reference strains *S. aureus*, *E. hirae*, *P. aeruginosa* and *E. coli* at the concentration/contact time relation **97 % / 5 min**.

Results are validated in accordance with the requirements of the EN 1276 (2009).

Greifswald, March 30, 2015

  
Dr. rer. med. (Dipl. Biol.) Torsten Koburger  
- General Manager -

  
Prof. Dr. med. A. Kramer  
MD for Hygiene and Environmental Medicine -

**Table 1: Results of the quantitative suspension test according to EN 1276 (2009)**

Date:	March 28, 2015	Order number:	A 15046
Product:	Sanitizer/Cleaner	Sample number:	P 150432
Test organism:	<i>S. aureus</i>	Lot number:	n.p.
Interfering substance:	0.03 % albumin	Neutralizer:	III
Incubation temperature:	36 ± 1 °C	Incubation time:	24 h - 48 h
Test suspension (N <sub>0</sub> ):	1.70*10 <sup>7</sup> cfu /ml (7.23 log)	Test temperature:	20 ± 1 °C
Validation Suspension (N <sub>v</sub> ):	3.50*10 <sup>7</sup> cfu/ml (2.54 log)		

contact time: 5 min									
concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V <sub>01</sub>	V <sub>02</sub>	log <sub>10</sub> Na	log <sub>10</sub> R
97 %	1 ml (10 <sup>7</sup> )	0	0	0	0	< 14	< 14	< 2.15	> 5.08
80 %	1 ml (10 <sup>7</sup> )	1	1	0	0	< 14	< 14	< 2.15	> 5.08
50 %	1 ml (10 <sup>7</sup> )	0	0	0	0	< 14	< 14	< 2.15	> 5.08
10 %	1 ml (10 <sup>7</sup> )	> 330	> 330	> 330	> 330	> 660	> 660	> 3.82	< 3.41

**Validation and Controls**

Validation - Suspension (N <sub>v</sub> )				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 97%			
cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$	cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$	cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$	cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$
V <sub>01</sub>	13	17	30	V <sub>01</sub>	24	26	50	V <sub>01</sub>	28	27	55	V <sub>01</sub>	28	21	49
V <sub>02</sub>	21	19	40	V <sub>02</sub>	21	30	51	V <sub>02</sub>	24	24	48	V <sub>02</sub>	26	29	55
30 ≤ $\bar{x}$ of N <sub>v</sub> ≤ 160?				$\bar{x}$ of A is ≥ 0.5* $\bar{x}$ of N <sub>v</sub> ?				$\bar{x}$ of B is ≥ 0.5* $\bar{x}$ of N <sub>v</sub> ?				$\bar{x}$ of C is ≥ 0.5* $\bar{x}$ of N <sub>v</sub> ?			
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			

**Table 2: Results of the quantitative suspension test according to EN 1276 (2009)**

Date:	March 28, 2015	Order number:	A 15046
Product:	Sanitizer/Cleaner	Sample number:	P 150432
Test organism:	<i>E. hirae</i>	Lot number:	n.p.
Interfering substance:	0.03 % albumin	Neutralizer:	III
Incubation temperature:	36 ± 1 °C	Incubation time:	24 h - 48 h
Test suspension (N <sub>0</sub> ):	1.50*10 <sup>7</sup> cfu /ml (7.18 log)	Test temperature:	20 ± 1 °C
Validation Suspension (N <sub>v</sub> ):	4.95*10 <sup>2</sup> cfu/ml (2.69 log)		

contact time: 5 min									
concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V <sub>c1</sub>	V <sub>c2</sub>	log <sub>10</sub> N <sub>a</sub>	log <sub>10</sub> R
97 %	1 ml (10 <sup>1</sup> )	0	0	0	0	< 14	< 14	< 2.15	> 5.03
80 %	1 ml (10 <sup>1</sup> )	0	0	0	0	< 14	< 14	< 2.15	> 5.03
50 %	1 ml (10 <sup>1</sup> )	12	22	18	14	34	32	2.52	4.66
10 %	1 ml (10 <sup>1</sup> )	> 330	> 330	> 330	> 330	> 660	> 660	> 3.82	< 3.36

**Validation and Controls**

Validation - Suspension (N <sub>0</sub> )				Experimental condition control (A)				Neutralizer control (B)				Method validation (C): Product concentration: 97%			
cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$	cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$	cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$	cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$
V <sub>c1</sub>	18	29	47	V <sub>c1</sub>	13	22	35	V <sub>c1</sub>	32	25	57	V <sub>c1</sub>	26	24	50
V <sub>c2</sub>	30	22	52	V <sub>c2</sub>	18	23	41	V <sub>c2</sub>	23	29	52	V <sub>c2</sub>	24	29	53
30 ≤ $\bar{x}$ of N <sub>0</sub> ≤ 160?				$\bar{x}$ of A is ≥ 0.5* $\bar{x}$ of N <sub>0</sub> ?				$\bar{x}$ of B is ≥ 0.5* $\bar{x}$ of N <sub>0</sub> ?				$\bar{x}$ of C is ≥ 0.5* $\bar{x}$ of N <sub>0</sub> ?			
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			

**Table 3: Results of the quantitative suspension test according to EN 1276 (2009)**

Date:	March 28, 2015	Order number:	A 15046
Product:	Sanitizer/Cleaner	Sample number:	P 150432
Test organism:	<i>P. aeruginosa</i>	Lot number:	n.p.
Interfering substance:	0.03 % albumin	Neutralizer:	III
Incubation temperature:	36 ± 1 °C	Incubation time:	24 h - 48 h
Test suspension (N <sub>t</sub> ):	1.90*10 <sup>7</sup> cfu /ml (7.28 log)	Test temperature:	20 ± 1 °C
Validation Suspension (N <sub>v</sub> ):	5.95*10 <sup>7</sup> cfu/ml (2.77 log)		

contact time: 5 min									
concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V <sub>d</sub>	V <sub>c</sub>	log <sub>10</sub> N <sub>a</sub>	log <sub>10</sub> R
97 %	1 ml (10 <sup>0</sup> )	0	0	0	0	< 14	< 14	< 2.15	> 5.13
80 %	1 ml (10 <sup>0</sup> )	0	0	0	0	< 14	< 14	< 2.15	> 5.13
50 %	1 ml (10 <sup>0</sup> )	18	20	17	20	38	37	2.57	4.70
10 %	1 ml (10 <sup>0</sup> )	> 330	> 330	> 330	> 330	> 660	> 660	> 3.82	< 3.46

**Validation and Controls**

Validation - Suspension (N <sub>v</sub> )				Experimental condition control (A)				Neutralizer control (B)				Method validation (C): Product concentration: 97%			
cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$	cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$	cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$	cfu / plate 1 & 2		V <sub>c</sub>	$\bar{x}$
V <sub>d</sub>	24	34	58	V <sub>d</sub>	51	43	94	V <sub>d</sub>	48	42	90	V <sub>d</sub>	24	25	49
V <sub>c</sub>	32	29	61	V <sub>c</sub>	42	49	91	V <sub>c</sub>	23	24	47	V <sub>c</sub>	21	31	52
30 ≤ x of N <sub>v</sub> ≤ 160?				x of A is ≥ 0.5* x of N <sub>v</sub> ?				x of B is ≥ 0.5* x of N <sub>v</sub> ?				x of C is ≥ 0.5* x of N <sub>v</sub> ?			
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			

**Table 4: Results of the quantitative suspension test according to EN 1276 (2009)**

Date:	March 28, 2015	Order number:	A 15046
Product:	Sanitizer/Cleaner	Sample number:	P 150432
Test organism:	<i>E. coli</i>	Lot number:	n.p.
Interfering substance:	0.03 % albumin	Neutralizer:	III
Incubation temperature:	36 ± 1 °C	Incubation time:	24 h - 48 h
Test suspension (N <sub>0</sub> ):	2.90*10 <sup>7</sup> cfu /ml (7.46 log)	Test temperature:	20 ± 1 °C
Validation Suspension (N <sub>v</sub> ):	8.80*10 <sup>7</sup> cfu/ml (2.94 log)		

contact time: 5 min									
concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V <sub>c1</sub>	V <sub>c2</sub>	log <sub>10</sub> N <sub>0</sub>	log <sub>10</sub> R
97 %	1 ml (10 <sup>7</sup> )	0	0	0	0	< 14	< 14	< 2.15	> 5.32
80 %	1 ml (10 <sup>6</sup> )	15	24	14	12	39	26	2.51	4.95
50 %	1 ml (10 <sup>5</sup> )	22	33	34	28	55	62	2.77	4.70
10 %	1 ml (10 <sup>4</sup> )	> 330	> 330	> 330	> 330	> 660	> 660	> 3.82	< 3.64

**Validation and Controls**

Validation - Suspension (N <sub>v0</sub> )				Experimental condition control (A)				Neutralizer control (B)				Method validation (C); Product concentration: 97%							
	cfu / plate 1 & 2	V <sub>c</sub>	$\bar{x}$		cfu / plate 1 & 2	V <sub>c</sub>	$\bar{x}$		cfu / plate 1 & 2	V <sub>c</sub>	$\bar{x}$		cfu / plate 1 & 2	V <sub>c</sub>	$\bar{x}$				
V <sub>c1</sub>	41	48	89	88	V <sub>c1</sub>	43	41	84	85	V <sub>c1</sub>	48	58	106	97.5	V <sub>c1</sub>	40	42	82	80.5
V <sub>c2</sub>	42	45	87		V <sub>c2</sub>	40	46	86		V <sub>c2</sub>	42	47	89		V <sub>c2</sub>	40	39	79	
30 ≤ $\bar{x}$ of N <sub>v0</sub> ≤ 160?				$\bar{x}$ of A is ≥ 0.5* $\bar{x}$ of N <sub>v0</sub> ?				$\bar{x}$ of B is ≥ 0.5* $\bar{x}$ of N <sub>v0</sub> ?				$\bar{x}$ of C is ≥ 0.5* $\bar{x}$ of N <sub>v0</sub> ?							
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no				<input checked="" type="checkbox"/> yes <input type="checkbox"/> no							

Legend:

MW	=	average value
x	=	average value
$\bar{x}$	=	average value
RF	=	reduction factor
R	=	reduction factor
> 330	=	not countable
> 660	=	not countable
n.d.	=	not determined
n.p.	=	not provided
WSH	=	water of standardized hardness