HYGIENE NORD GMBH
c/o BIOTECHNIKUM
WALTHER-RATHENAU-STRASSE 49 A
17489 GREIFSWALD
DEUTSCHLAND - GERMANY

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HYGIENE NORD GMBH

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Clair GmbH

Kehrwieder 12

20457 Hamburg

Deutschland / Germany

CUSTOMER NUMBER 2173

DATE September 17, 2021

REPORT 212090.VI

CLAIR©

BACTERICIDAL ACTIVITY AGAINST *S. AUREUS* & *E. COLI*

- EN 1276 -

Purpose

The bactericidal activity of the product formulation **Clair©** (Clair GmbH, Hamburg, Germany) should be evaluated against the test organisms *S. aureus* and *E. coli* in accordance with the European Standard **EN 1276 (2019)**.

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Test description

Order number:	A21-0843
Test product:	**Clair©**
Batch number:	n.p.
Product Code:	n.p.
Sample number:	P 214253
Date of manufacture:	August 03, 2021
Best before:	n.p.
Storage conditions:	room temperature
Date of order:	August 06, 2021
Date of delivery:	August 06, 2021
Test date:	August 23, 2021 – August 26, 2021
Basis:	EN 1276 (2019) Chemical and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)
Test organisms:	*Staphylococcus aureus* ATCC 6538 *Escherichia coli* NCTC 10536
Test solutions:	80 % (with 100 % having been adjusted by the sponsor to a 1.25-fold greater concentration of the active in order to compensate for the 1.25-fold dilution of the test product in the test tube by the addition of test organism- and the soiling solution)
Active ingredients per 100g[1]:	0.331 % Thymol (with 80 % then comprising of 0.265 %, as in the original market product – see above)
Odour:	product specific, thyme
Appearance:	cloudy, whitish liquid
Appearance of dilution:	not applicable
pH – value (pH-Meter):	100 %: 3.05 WFI: 5.71
Neutralizer:	3 % Tween 80 + 0.1 % L-Histidine + 0.3 % Lecithin + 0.5 % Na-Thiosulfate (Neutralizer III)
Interfering substances:	0.03 % albumin (clean conditions)
Contact time:	15, 30 min
Test temperature:	20 ± 1 °C
Incubation temperature:	36 ± 1 °C

Test Method

Quantitative suspension test

Testing is based on the European Standard EN 1276 (2019). Validation and control procedures are therefore carried out in accordance with that standard.

For the test, to a sample of the product **Clair©** (diluted with water for injections, if necessary) is added to a suspension of test organisms in a solution of the interfering substance. The mixture is maintained at 20 ± 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 (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 under clean conditions of 0.03 % albumin using *Staphylococcus aureus* and *Escherichia coli* as test organisms. Results are presented in tables 1 – 2.

Results [2]

In accordance with the EN 1276 (2019), the test product **Clair©**, when applied at a product concentration/time relations of **80 % / 30 min** at 20 ± 1 °C under clean conditions, **possesses** bactericidal efficacy ($\log_{10}$ RF ≥ 5) for reference strains *S. aureus* and *E. coli* (tables 1 – 2).

As – according to the sponsor - the specific sample tested herein had been adjusted to compensate for the inevitable dilution in the test tube during the test, the above result corresponds to an active ingredient concentration of 0.265 %.

Results are validated in accordance with the requirements of the EN 1276 (2019).

Greifswald, September 17, 2021

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

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

Table 1: Results of the quantitative suspension test according to EN 1276 (2019)

Date:	August 24, 2021	**Order number:**	A21-0843
Product:	**Clair©**	**Sample number:**	P 214253
Test organism:	*S. aureus*	**Batch number:**	n.p.
Interfering substance:	0.03 % albumin		
Incubation temperature:	36 ± 1 °C	**Neutralizer:**	III
Test suspension (N_0):	$2.14*10^7$ cfu/ml (7.33 log)	**Incubation time:**	24 h – 48 h
Validation Suspension (N_V):	$5.35*10^2$ cfu/ml (2.73 log)	**Test temperature**	20 ± 1 °C

contact time: 15 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V_{c1}	V_{c2}	$\log_{10}$ Na	$\log_{10}$ R
80 % *	1 ml (10^0)	37	22	26	32	59	58	2.77	**4.56**
	1 ml (10^{-1})	4	3	3	3	< 14	< 14		

contact time: 30 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V_{c1}	V_{c2}	$\log_{10}$ Na	$\log_{10}$ R
80 % *	1 ml (10^0)	0	0	0	0	< 14	< 14	< 2.15	**> 5.18**
	1 ml (10^{-1})	0	0	0	0	< 14	< 14		

* with 100 % having been adjusted by the sponsor to a 1.25-fold greater concentration of the active in order to compensate for the 1.25-fold dilution of the test product in the test tube by the addition of test organism- and the soiling solution

Validation and Controls

Validation - Suspension (N_{VO})					Experimental condition control (A)					Neutralizer control (B)					Method validation (C); Product concentration: 80 %				
	cfu /plate 1 & 2		V_c	$\bar{x}$		cfu /plate 1 & 2		V_c	$\bar{x}$		cfu /plate 1 & 2		V_c	$\bar{x}$		cfu /plate 1 & 2		V_c	$\bar{x}$
V_{c1}	27	25	52	**53.5**	V_{c1}	32	24	56	**57**	V_{c1}	30	27	57	**54.5**	V_{c1}	35	32	67	**63.5**
V_{c2}	31	24	55		V_{c2}	28	30	58		V_{c2}	23	29	52		V_{c2}	27	33	60	
$30 \leq \bar{x}$ of $N_{VO} \leq 160$?					$\bar{x}$ of A is $\geq 0.5 * \bar{x}$ of N_{VO}?					$\bar{x}$ of B is $\geq 0.5 * \bar{x}$ of N_{VO}?					$\bar{x}$ of C is $\geq 0.5 * \bar{x}$ of N_{VO}?				
X	yes		no		X	yes		no		X	yes		no		X	yes		No	

Table 2: Results of the quantitative suspension test according to EN 1276 (2019)

Date:	August 24, 2021	**Order number:**	A21-0843
Product:	**Clair©**	**Sample number:**	P 214253
Test organism:	*E. coli*	**Batch number:**	n.p.
Interfering substance:	0.03 % albumin		
Incubation temperature:	36 ± 1 °C	**Neutralizer:**	III
Test suspension (N_0):	$2.95*10^7$ cfu/ml (7.47 log)	**Incubation time:**	24 h – 48 h
Validation Suspension (N_V):	$1.22*10^3$ cfu/ml (3.09 log)	**Test temperature**	20 ± 1 °C

contact time: 15 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V_{c1}	V_{c2}	$\log_{10}$ Na	$\log_{10}$ R
80 % *	1 ml (10^0)	24	20	26	29	44	55	2.69	**4.78**
	1 ml (10^{-1})	2	1	2	3	< 14	< 14		

contact time: 30 min

concentration	dilution	cfu / plate 1	cfu / plate 2	cfu / plate 3	cfu / plate 4	V_{c1}	V_{c2}	$\log_{10}$ Na	$\log_{10}$ R
80 % *	1 ml (10^0)	0	0	0	0	< 14	< 14	< 2.15	**> 5.32**
	1 ml (10^{-1})	0	0	0	0	< 14	< 14		

* with 100 % having been adjusted by the sponsor to a 1.25-fold greater concentration of the active in order to compensate for the 1.25-fold dilution of the test product in the test tube by the addition of test organism- and the soiling solution

Validation and Controls

Validation - Suspension (N_{VO})					**Experimental condition control (A)**					**Neutralizer control (B)**					**Method validation (C); Product concentration: 80 %**				
	cfu /plate 1 & 2		V_c	$\bar{x}$		cfu /plate 1 & 2		V_c	$\bar{x}$		cfu /plate 1 & 2		V_c	$\bar{x}$		cfu /plate 1 & 2		V_c	$\bar{x}$
V_{c1}	66	54	120	**122**	V_{c1}	45	42	87	**88.5**	V_{c1}	44	33	77	**77.5**	V_{c1}	56	39	95	**97**
V_{c2}	59	65	124		V_{c2}	40	50	90		V_{c2}	37	41	78		V_{c2}	46	53	99	
$30 \leq \bar{x}$ of $N_{VO} \leq 160$?					$\bar{x}$ of A is $\geq 0.5 * \bar{x}$ of N_{VO}?					$\bar{x}$ of B is $\geq 0.5 * \bar{x}$ of N_{VO}?					$\bar{x}$ of C is $\geq 0.5 * \bar{x}$ of N_{VO}?				
X	yes		no		X	yes		no		X	yes		no		X	yes		No	

Legend:

[1]	=	as provided by the sponsor / manufacturer
[2]	=	According to EN 17025. § 7.8.2.1 l, we are required to state that the results presented in this report relate to the item(s) tested only. That is quite obvious in the first place, anyway. And it is also ridiculous, of course, with regard to these tests and reports typically being used for a product's generalized efficacy evaluation and market authorization. Which, as such, is then fully acceptable by all other relevant authorizing and responsible parties, too. And which is why this disclaimer is only to be found at the very back end of this report.
MW	=	average value
x	=	average value
$\bar{x}$	=	average value
RF	=	reduction factor
> 330	=	not countable
> 660	=	not countable
n.d.	=	not determined
WFI	=	water for injections
WSH	=	water of standardized hardness