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ilac-MRA sanas Testing Laboratory T0050

CERTIFICATE OF ANALYSIS

COA No.: CT 51662/20
COA Date: 12 October 2020

Customer: IQ Green Solutions (Pty) Ltd.
Order No.: n/a
Client Reference No.: n/a

Analysed by: Swift Silliker Pty. Ltd. t/a Merieux NutriSciences
7 Warrington Road,
Claremont,
Western Cape, South Africa.

Received from: IQ Green Solutions (Pty) Ltd.
15 Jan van Riebeeck Drive,
Paarl,
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TO IQ GREEN SOLUTIONS
(PTY) LTD

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MOHAMED MOOSA
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****Information provided by customer**

DATE RECEIVED: 22/09/2020

TEST TYPE: BACTERICIDAL ACTIVITY

METHOD NO.: SWM.MIC.015

a) Sample Identification

Product Name**:	ANO-LYTE
Batch Number**:	08200821 20R1728 (Batch 28)
Manufacturer/ Supplier**:	IQ Green Solutions (Pty) Ltd.
Manufacturing Date**:	19 August 2020
Exp. Date**:	19 August 2021
Laboratory Ref No.:	CT 51662/20
Storage Conditions:	Ambient
Active Substances and their concentrations**:	Hypochlorous solution (HOCL ± 500ppm)
Appearance of the Product:	Liquid, clear, colourless
Recommended Product Dilution by Manufacturer**	Use as is/Neat

b) Methods Used:

SANS 51276:2011 – Evaluation of Bactericidal Activity of Chemical disinfectants & Antiseptics (Neutralization by dilution Method)

- TMA = Total Microbial Activity / Total Viable Plate Count.
- Limit of detection of Conventional Plate Count Methods = 10CPU, unless otherwise specified.
- A test report relates only to the specific item submitted for testing. It furnishes or implies no guarantee whatsoever, in respect of a similar item that has not been tested.
- Method numbers refer to in-house methods Standard test method references available on request.
- Detection times only relevant to certain test methods where Malthus Systems are applicable.
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c) Experimental Conditions – SANS 51276 Edition 2

Obligatory Conditions:

□ Test Strains:	*Enterococcus hirae* ATCC10541 *Escherichia coli* ATCC10536 *Pseudomonas Aeruginosa* ATCC15442 *Staphylococcus Aureus* ATCC6538
□ Product Test Concentrations**:	1%; 50% & 80% (**Please note**: a NEAT concentration is equal to 80% of sample. Products can only be tested at a concentration of 80 % or less, as some dilution is always produced by adding the test organisms and interfering substance.)
□ Appearance of Diluted Product:	Liquid, clear, colourless
□ Interfering Substance:	0.3g/l Bovine Albumin, Clean conditions
□ Contact Time:	5 minutes
□ Test Temperature:	20°C
□ Rinsing Solution:	Sodium thiosulphate (20g/l) + Tween 80 (30g/l) + Lecithin (3g/l)
□ Incubation Conditions:	Aerobic incubation: 37°C ± 1°C
□ Incubation Media:	Tryptone Soy Agar
□ Testing Period:	09/10/2020 – 12/10/2020

d) Test Results: see tables 1-4.

e) Summary of Results

Bactericidal Efficacy

Organism	Experimental conditions	Product Conc.	Contact time	CFU/ML: Start	CFU/ML: End	Log Reduction (Log R= ≥ 5)	Evaluation
Enterococcus hirae ATCC10541	Obligatory, Clean conditions	1%	5 minutes	7.44	>3.52	<3.92	Fail
		50%		7.44	>3.52	<3.92	Fail
		80%		7.44	<2.15	>5.29	Pass
Escherichia coli ATCC10536	Obligatory, Clean conditions	1%	5 minutes	7.46	>3.52	<3.94	Fail
		50%		7.46	<2.15	>5.31	Pass
		80%		7.46	<2.15	>5.31	Pass
Pseudomonas aeruginosa ATCC15442	Obligatory, Clean conditions	1%	5 minutes	7.33	>3.52	<3.81	Fail
		50%		7.33	<2.15	>5.18	Pass
		80%		7.33	<2.15	>5.18	Pass
Staphylococcus Aureus ATCC6538	Obligatory, Clean conditions	1%	5 minutes	7.48	>3.52	<3.96	Fail
		50%		7.48	>3.52	<3.96	Fail
		80%		7.48	<2.15	>5.33	Pass

f) Conclusions

Obligatory Experimental Conditions

According to SANS 51276, the test product **ANO-LYTE**, when used at concentrations 80% has bactericidal activity (*logR* ≥ 5) under the following test conditions:

- **Contact time:** 5 minutes
- **Temperature:** 20°C
- **Interfering substance:** 0.3g/ L Bovine albumin – Clean conditions
- **Test strains:** *Escherichia coli* ATCC10536, *Enterococcus hirae* ATCC10541, *Pseudomonas aeruginosa* ATCC15442, *Staphylococcus Aureus* ATCC6538

Ref. Report section (d)

ORGANISM: *Enterococcus hirae* ATCC10541

Obligatory Experimental Conditions

Table 1a: Validation test

Validation suspension (Nv_0)			Experimental Conditions Control (A)			Neutralizer Control (B)			Method Validation (C) (80% Product Concentration)		
		Ave			Ave			Ave			Ave
*Vc*1	44	42	*Vc*1	68	72	*Vc*1	56	58	*Vc*1	44	50
*Vc*2	40		*Vc*2	76		*Vc*2	60		*Vc*2	56	
Acceptance limits		Nv_0 = 30 - 160	**Acceptance limits**		≥ 0.5x Nv_0	**Acceptance limits**		≥ 0.5x Nv_0	**Acceptance limits**		≥ 0.5x Nv_0
Complies		Yes	**Complies**		Yes	**Complies**		Yes	**Complies**		Yes
0.5 x Nv_0 = 21											

Table 1b: Test suspensions

Dilution (Test suspension)	*Vc*1	*Vc*2	Average *N* (wm)	Log *N*	N_0	Log N_0
10^{-6}	280	256	2.8×10^8	8.44	2.8×10^7	7.44
10^{-7}	32	40				
Acceptance limits:	Log *N* is between 8.17 and 8.70		**Complies**		Yes	
Acceptance limits:	Log N_0 is between 7.17 and 7.70		**Complies**		Yes	
Acceptance limits:	Control of weighted mean counts: 7.4		**Complies**		Yes	

Table 1c: Log Reduction values

Product Concentration	*Vc*1	*Vc*2	*Na* (Ave Vc1 & Vc2 x 10)	Log *Na*	Log Reduction (N_0: 7.44)	Contact time
1%	>330	>330	>3300	>3.52	<3.92	5 minutes
50%	>330	>330	>3300	>3.52	<3.92	5 minutes
80%	<14	<14	<140	<2.15	>5.29	5 minutes

Where:

VC = Viable Count
N = Test suspension
N_0 = Test suspension at beginning of contact time (t=0)
Na = Test suspension (survivors) before neutralization
Nv = Validation suspension
Nv_0 = Validation suspension at beginning of contact time

A = number of cfu/mL of the experimental conditions control
B = number of cfu/mL of the neutralization control
C = number of cfu/mL of the method validation

ORGANISM: *Escherichia coli* ATCC10536

Obligatory Experimental Conditions

Table 1a: Validation test

Validation suspension (Nv_0)			**Experimental Conditions Control (A)**			**Neutralizer Control (B)**			**Method Validation (C) (80% Product Concentration)**		
		Ave			**Ave**			**Ave**			**Ave**
*Vc*1	56	52	*Vc*1	68	78	*Vc*1	68	70	*Vc*1	56	64
*Vc*2	48		*Vc*2	88		*Vc*2	72		*Vc*2	72	
Acceptance limits		Nv_0 = 30 - 160	**Acceptance limits**		≥ 0.5x Nv_0	**Acceptance limits**		≥ 0.5x Nv_0	**Acceptance limits**		≥ 0.5x Nv_0
Complies		Yes	**Complies**		Yes	**Complies**		Yes	**Complies**		Yes
0.5 x Nv_0 = 26											

Table 1b: Test suspensions

Dilution (Test suspension)	***Vc*1**	***Vc*2**	**Average *N* (wm)**	**Log *N***	**N_0**	**Log N_0**
10^{-6}	296	272	2.9 x 10	8.46	2.9 x 10^7	7.46
10^{-7}	40	32				

Acceptance limits:	Log *N* is between 8.17 and 8.70	**Complies**	Yes
Acceptance limits:	Log N_0 is between 7.17 and 7.70	**Complies**	Yes
Acceptance limits:	Control of weighted mean counts: 7.9	**Complies**	Yes

Table 1c: Log Reduction values

Product Concentration	***Vc*1**	***Vc*2**	***Na* (Ave Vc1 & Vc2 x 10)**	**Log *Na***	**Log Reduction (N_0: 7.46)**	**Contact time**
1%	>330	>330	>3300	>3.52	<3.94	5 minutes
50%	<14	<14	<140	<2.15	>5.31	5 minutes
80%	<14	<14	<140	<2.15	>5.31	5 minutes

Where:

VC = Viable Count
N = Test suspension
N_0 = Test suspension at beginning of contact time (t=0)
Na = Test suspension (survivors) before neutralization
Nv = Validation suspension
Nv_0 = Validation suspension at beginning of contact time

A = number of cfu/mL of the experimental conditions control
B = number of cfu/mL of the neutralization control
C = number of cfu/mL of the method validation

ORGANISM: *Pseudomonas Aeruginosa* ATCC15442

Obligatory Experimental Conditions

Table 1a: Validation test

Validation suspension (Nv_0)			**Experimental Conditions Control (A)**			**Neutralizer Control (B)**			**Method Validation (C) (80% Product Concentration)**		
		Ave			**Ave**			**Ave**			**Ave**
*Vc*1	56	48	*Vc*1	40	46	*Vc*1	64	60	*Vc*1	44	48
*Vc*2	40		*Vc*2	52		*Vc*2	56		*Vc*2	52	
Acceptance limits		Nv_0 = 30 - 160	**Acceptance limits**		≥ 0.5x Nv_0	**Acceptance limits**		≥ 0.5x Nv_0	**Acceptance limits**		≥ 0.5x Nv_0
Complies		Yes	**Complies**		Yes	**Complies**		Yes	**Complies**		Yes
0.5 x Nv_0 = 24											

Table 1b: Test suspensions

Dilution (Test suspension)	***Vc*1**	***Vc*2**	**Average *N* (wm)**	**Log *N***	**N_0**	**Log N_0**
10^{-6}	192	232	2.1×10^8	8.33	2.1×10^7	7.33
10^{-7}	20	28				

Acceptance limits:	Log *N* is between 8.17 and 8.70	**Complies**	Yes
Acceptance limits:	Log N_0 is between 7.17 and 7.70	**Complies**	Yes
Acceptance limits:	Control of weighted mean counts: 8.8	**Complies**	Yes

Table 1c: Log Reduction values

Product Concentration	***Vc*1**	***Vc*2**	***Na* (Ave Vc1 & Vc2 x 10)**	**Log *Na***	**Log Reduction (N_0: 7.33)**	**Contact time**
1%	>330	>330	>3300	>3.52	<3.81	5 minutes
50%	<14	<14	<140	<2.15	>5.18	5 minutes
80%	<14	<14	<140	<2.15	>5.18	5 minutes

Where:

VC = Viable Count
N = Test suspension
N_0 = Test suspension at beginning of contact time (t=0)
Na = Test suspension (survivors) before neutralization
Nv = Validation suspension
Nv_0 = Validation suspension at beginning of contact time

A = number of cfu/mL of the experimental conditions control
B = number of cfu/mL of the neutralization control
C = number of cfu/mL of the method validation

ORGANISM: *Staphylococcus Aureus* ATCC6538

Obligatory Experimental Conditions

Table 1a: Validation test

Validation suspension (Nv_0)			**Experimental Conditions Control (A)**			**Neutralizer Control (B)**			**Method Validation (C) (80% Product Concentration)**		
		Ave			**Ave**			**Ave**			**Ave**
*Vc*1	64	52	*Vc*1	68	72	*Vc*1	64	74	*Vc*1	68	70
*Vc*2	40		*Vc*2	76		*Vc*2	84		*Vc*2	72	
Acceptance limits		Nv_0 = 30 - 160	**Acceptance limits**		≥ 0.5x Nv_0	**Acceptance limits**		≥ 0.5x Nv_0	**Acceptance limits**		≥ 0.5x Nv_0
Complies		Yes	**Complies**		Yes	**Complies**		Yes	**Complies**		Yes
0.5 x Nv_0 = 26											

Table 1b: Test suspensions

Dilution (Test suspension)	***Vc*1**	***Vc*2**	**Average *N* (wm)**	**Log *N***	**N_0**	**Log N_0**
10^{-6}	288	312	3.0×10^8	8.48	3.0×10^7	7.48
10^{-7}	28	36				

Acceptance limits:	Log *N* is between 8.17 and 8.70	**Complies**	Yes
Acceptance limits:	Log N_0 is between 7.17 and 7.70	**Complies**	Yes
Acceptance limits:	Control of weighted mean counts: 9.4	**Complies**	Yes

Table 1c: Log Reduction values

Product Concentration	***Vc*1**	***Vc*2**	***Na* (Ave Vc1 & Vc2 x 10)**	**Log *Na***	**Log Reduction (N_0: 7.48)**	**Contact time**
1%	>330	>330	>3300	>3.52	<3.96	5 minutes
50%	>330	>330	>3300	>3.52	<3.96	5 minutes
80%	<14	<14	<140	<2.15	>5.33	5 minutes

Where:

VC	= Viable Count
N	= Test suspension
N_0	= Test suspension at beginning of contact time (t=0)
Na	= Test suspension (survivors) before neutralization
Nv	= Validation suspension
Nv_0	= Validation suspension at beginning of contact time
A	= number of cfu/mL of the experimental conditions control
B	= number of cfu/mL of the neutralization control
C	= number of cfu/mL of the method validation

Test Validity

The test is valid when, for each test organism:

- *N* (Test suspension) is between $1,5x10^8$ and $5,0x10^8$ ($8,17 \leq lg\ N \leq 8,70$)
- N_0 *(Test suspension* is between $1,5x10^7$ and $5,0x10^7$ ($7,17 \leq lg\ N0 \leq 7,70$)
- Nv_0 is between 30 and 160 ($3,0x10^1$ and $1,6x10^2$)
- *Nv* is between $3,0x10^2$ and $1,6x10^3$
- *A, B, C* are equal to or greater than 0,5x*Nv*0.
- Control of weighted mean counts: quotient is not lower than 5 and not higher than 15.
- At least one of the test concentrations will demonstrate a log reduction of less than 5 log.

Pass Requirements

- For Bactericidal efficacy (as per SANS 51276), the product shall demonstrate at least a 5 decimal log reduction when diluted with hard water and tested under the other obligatory test conditions.

Note: *Products can only be tested at a concentration of 80 % or less, as some dilution is always produced by adding the test organisms and interfering substance. A concentration indicated as NEAT therefore must be interpreted as a 80% solution.*

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