ilac-MRA sanas Testing Laboratory T0050

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## CERTIFICATE OF ANALYSIS

**COA No.:** CT 49159/20
**COA Date:** 11 May 2020
**Page:** 1/6

**Customer:** The Bio Consulting Pty Ltd t/a Biorevolution
**Order No.:** n/a
**Client Reference No.: n/a**
**Project No.:** CT 49159/20

**Analysed by:** Swift Silliker Pty. Ltd. t/a Merieux NutriSciences
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****Information provided by customer**

**DATE RECEIVED:** 22/04/2020

**TEST TYPE:** DISINFECTANT – BACTERICIDAL ACTIVITY (SANS 51276:2011)

**METHOD NO.:** SWM.MIC.015

**a) Sample Identification**

| | |
|---|---|
| □ Product Name**: | ORGANIC FRESH |
| □ Active Ingredient**: | Bioflavenoid Mixture |
| □ Batch Number**: | n/a |
| □ Manufacturing Date**: | n/a |
| □ Exp. Date**: | n/a |
| □ Laboratory Number: | CT 49159/20 |
| □ Storage Conditions: | Ambient |
| □ Appearance: | Liquid, Dark, Brown |

**b) Methods Used:**

□ EN 1276:2011 – Evaluation of Bactericidal Activity
(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.
- 

**c) Experimental Conditions**

| | |
|---|---|
| ☐ Test Strains: | *Escherichia coli* ATCC10536<br>*Enterococcus hirae* ATCC10541<br>*Pseudomonas Aeruginosa* ATCC15442<br>*Staphylococcus Aureus* ATCC6538 |
| ☐ Product Test Concentrations**: | 0.1%, 0.5% & 1.5% |
| ☐ Appearance of Diluted Product: | 0.1% - liquid, clear, colourless<br>0.5% - liquid, clear, colourless<br>1.5% - liquid, clear, brown |
| ☐ Interfering Substance: | 0.3g/l Bovine Albumin, Clean conditions |
| ☐ Contact Time: | 5 Minutes |
| ☐ Test Temperature: | 20°C |
| ☐ Neutraliser: | Tween 80 (30g/l) + Saponin (30g/l) + Lecithin (3g/l) |
| ☐ Incubation Conditions: | Aerobic incubation: 37°C ± 1°C |
| ☐ Incubation Media: | Tryptone Soy Agar |
| ☐ Testing Period: | 05/05 – 08/05/2020 |

**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 | 0.1% | 5 minutes | 7.39 | >3.52 | <3.87 | Fail |
| | | 0.5% | | 7.39 | <2.15 | >5.24 | Pass |
| | | 1.5% | | 7.39 | <2.15 | >5.24 | Pass |
| *Escherichia coli* ATCC10536 | Obligatory, Clean conditions | 0.1% | 5 minutes | 7.32 | >3.52 | <3.80 | Fail |
| | | 0.5% | | 7.32 | <2.15 | >5.17 | Pass |
| | | 1.5% | | 7.32 | <2.15 | >5.17 | Pass |
| *Pseudomonas aeruginosa* ATCC15442 | Obligatory, Clean conditions | 0.1% | 5 minutes | 7.26 | >3.52 | <3.74 | Fail |
| | | 0.5% | | 7.26 | <2.15 | >5.11 | Pass |
| | | 1.5% | | 7.26 | <2.15 | >5.11 | Pass |
| *Staphylococcus Aureus* ATCC6538 | Obligatory, Clean conditions | 0.1% | 5 minutes | 7.33 | >3.52 | <3.81 | Fail |
| | | 0.5% | | 7.33 | <2.15 | >5.18 | Pass |
| | | 1.5% | | 7.33 | <2.15 | >5.18 | Pass |

**f) Conclusions**

According to SANS 51276, the test product, **ORGANIC FRESh** when used at concentrations 0.5% & 1.5% 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* ATCC 10536, *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)= NvA** | | | **Neutralizer Control (B)= NvB** | | | **Method Validation (C) (1.5% Product Concentration)= NvC** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Ave** | | | **Ave** | | | **Ave** | | | **Ave** |
| *Vc*1 | 44 | 38 | *Vc*1 | 46 | 43 | *Vc*1 | 48 | 42 | *Vc*1 | 33 | 35 |
| *Vc*2 | 32 | | *Vc*2 | 40 | | *Vc*2 | 36 | | *Vc*2 | 37 | |
| **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 $Nv_0$ = 19 | | | | | | | | | | | |

**Table 1b: Test suspensions**

| **Dilution (Test suspension)** | ***Vc*1** | ***Vc*2** | **Average *N* (wm)** | **Log *N*** | **$N_0$** | **Log $N_0$** |
|---|---|---|---|---|---|---|
| $10^{-6}$ | 240 | 224 | $2.4 \times 10^8$ | 8.39 | $2.4 \times 10^7$ | 7.39 |
| $10^{-7}$ | 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: 6.4 | | **Complies** | | Yes | |

**Table 1c: Log Reduction values**

| **Product Concentration** | ***Vc*1** | ***Vc*2** | ***Na* (Ave Vc1 & Vc2 x 10)** | **Log *Na*** | **Log Reduction ($N_0$: 7.39)** | **Contact time** |
|---|---|---|---|---|---|---|
| 0.1% | >330 | >330 | >3300 | >3.52 | <3.87 | 5 Minutes |
| 0.5% | <14 | <14 | <140 | <2.15 | >5.24 | 5 Minutes |
| 1.5% | <14 | <14 | <140 | <2.15 | >5.24 | 5 Minutes |

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## ORGANISM: *Escherichia coli* ATCC 10536

**Table 2a: Validation test**

| **Validation suspension (*Nv0*)** | | | **Experimental Conditions Control (A)= NvA** | | | **Neutralizer Control (B)= NvB** | | | **Method Validation (C) (1.5% Product Concentration)= NvC** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Ave** | | | **Ave** | | | **Ave** | | | **Ave** |
| *Vc*1 | 34 | 35.5 | *Vc*1 | 33 | 31.5 | *Vc*1 | 40 | 39 | *Vc*1 | 29 | 29.5 |
| *Vc*2 | 37 | | *Vc*2 | 30 | | *Vc*2 | 38 | | *Vc*2 | 30 | |
| **Acceptance limits** | | $Nv_0$ = 30 - 160 | **Acceptance limits** | | ≥ 0.5x *Nv0* | **Acceptance limits** | | ≥ 0.5x *Nv0* | **Acceptance limits** | | ≥ 0.5x *Nv0* |
| **Complies** | | Yes | **Complies** | | Yes | **Complies** | | Yes | **Complies** | | Yes |
| 0.5 x $Nv_0$ = 17.75 | | | | | | | | | | | |

**Table 2b: Test suspensions**

| **Dilution (Test suspension)** | ***Vc*1** | ***Vc*2** | **Average *N* (wm)** | **Log *N*** | ***N0*** | **Log *N0*** |
|---|---|---|---|---|---|---|
| $10^{-6}$ | 208 | 192 | $2.1 \times 10^8$ | 8.32 | $2.1 \times 10^7$ | 7.32 |
| $10^{-7}$ | 28 | 34 | | | | |

| | | | |
|---|---|---|---|
| **Acceptance limits:** | Log *N* is between 8.17 and 8.70 | **Complies** | Yes |
| **Acceptance limits:** | Log *N0* is between 7.17 and 7.70 | **Complies** | Yes |
| **Acceptance limits:** | Control of weighted mean counts: 6.5 | **Complies** | Yes |

**Table 2c: Log Reduction values**

| **Product Concentration** | ***Vc*1** | ***Vc*2** | ***Na* (Ave Vc1 & Vc2 x 10)** | **Log *Na*** | **Log Reduction (*N0*: 7.32)** | **Contact time** |
|---|---|---|---|---|---|---|
| 0.1% | >330 | >330 | >3300 | >3.52 | <3.80 | 5 Minutes |
| 0.5% | <14 | <14 | <140 | <2.15 | >5.17 | 5 Minutes |
| 1.5% | <14 | <14 | <140 | <2.15 | >5.17 | 5 Minutes |

## ORGANISM: *Pseudomonas Aeruginosa* ATCC15442

**Table 3a: Validation test**

| **Validation suspension (*Nv0*)** | | | **Experimental Conditions Control (A)= NvA** | | | **Neutralizer Control (B)= NvB** | | | **Method Validation (C) (1.5% Product Concentration)= NvC** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Ave** | | | **Ave** | | | **Ave** | | | **Ave** |
| *Vc*1 | 36 | 38 | *Vc*1 | 30 | 34.5 | *Vc*1 | 48 | 52 | *Vc*1 | 25 | 27 |
| *Vc*2 | 40 | | *Vc*2 | 39 | | *Vc*2 | 56 | | *Vc*2 | 29 | |
| **Acceptance limits** | | $Nv_0$ = 30 - 160 | **Acceptance limits** | | ≥ 0.5x *Nv0* | **Acceptance limits** | | ≥ 0.5x *Nv0* | **Acceptance limits** | | ≥ 0.5x *Nv0* |
| **Complies** | | Yes | **Complies** | | Yes | **Complies** | | Yes | **Complies** | | Yes |
| 0.5 x $Nv_0$ = 19 | | | | | | | | | | | |

| | |
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| **COA Date:** | 11/05/2020 |
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**Table 3b: Test suspensions**

| Dilution (Test suspension) | *Vc*1 | *Vc*2 | Average *N* (wm) | Log *N* | *N*₀ | Log *N*₀ |
|---|---|---|---|---|---|---|
| $10^{-6}$ | 160 | 176 | $1.8 \times 10^8$ | 8.26 | $1.8 \times 10^7$ | 7.26 |
| $10^{-7}$ | 30 | 33 | | | | |
| | | | | | | |
| **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: 5.3 | | **Complies** | | Yes | |

**Table 3c: Log Reduction values**

| Product Concentration | *Vc*1 | *Vc*2 | *Na* (Ave Vc1 & Vc2 x 10) | Log *Na* | Log Reduction (*N*₀: 7.26) | Contact time |
|---|---|---|---|---|---|---|
| 0.1% | >330 | >330 | >3300 | >3.52 | <3.74 | 5 Minutes |
| 0.5% | <14 | <14 | <140 | <2.15 | >5.11 | 5 Minutes |
| 1.5% | <14 | <14 | <140 | <2.15 | >5.11 | 5 Minutes |

## ORGANISM: *Staphylococcus Aureus* ATCC6538

**Table 4a: Validation test**

| Validation suspension (*Nv*₀) | | | Experimental Conditions Control (A)= NvA | | | Neutralizer Control (B)= NvB | | | Method Validation (C) (1.5% Product Concentration)= NvC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ave | | | Ave | | | Ave | | | Ave |
| *Vc*1 | 56 | 52 | *Vc*1 | 38 | 36.5 | *Vc*1 | 26 | 31 | *Vc*1 | 32 | 33 |
| *Vc*2 | 48 | | *Vc*2 | 35 | | *Vc*2 | 36 | | *Vc*2 | 34 | |
| **Acceptance limits** | | Nv₀ = 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 Nv₀ = 26 | | | | | | | | | | | |

**Table 4b: Test suspensions**

| Dilution (Test suspension) | *Vc*1 | *Vc*2 | Average *N* (wm) | Log *N* | *N*₀ | Log *N*₀ |
|---|---|---|---|---|---|---|
| $10^{-6}$ | 184 | 216 | $2.1 \times 10^8$ | 8.33 | $2.1 \times 10^7$ | 7.33 |
| $10^{-7}$ | 36 | 30 | | | | |
| | | | | | | |
| **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: 6.1 | | **Complies** | | Yes | |

**Table 4c: 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 |
|---|---|---|---|---|---|---|
| 0.1% | >330 | >330 | >3300 | >3.52 | <3.81 | 5 Minutes |
| 0.5% | <14 | <14 | <140 | <2.15 | >5.18 | 5 Minutes |
| 1.5% | <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**

**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.

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