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T0050

CERTIFICATE OF ANALYSIS

COA No.: GT 114795/20

COA Date: 02/09/2020

Analysed By: Swift Silliker Pty. Ltd. t/a Merieux NutriSciences
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DATE RECEIVED: 14/08/2020

TEST TYPE: Chemical Disinfectants and Sanitisers- Bactericidal Activity (SANS 51276: 2011)

METHOD NO.: SWM.MIC.015

a) Sample Identification:

□ Product Name:	Hand 'n Sani Liquid Hand Sanitizer
□ Laboratory Number:	GT 114795/20
□ Active substances and their concentrations:	Ethyl Alcohol
□ Appearance of the product:	Clear Liquid

b) Methods Used:

□ SANS 51276 – Evaluation of Bactericidal Activity of chemical disinfectants and 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:	*Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541
□ Product Test Concentrations:	10%,50% and Neat
□ Appearance of Diluted Product:	Clear Liquid
□ Interfering Substance:	0.3g/l Bovine Albumin, Clean Conditions
□ Contact Time:	1 Minute
□ Test Temperature:	20 °C
□ Neutralizer Solution:	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:	27-28/08/2020

d) Test Results: See tables 1-4

e) Summary of results:

Bactericidal Efficacy:

Organism	Experimental conditions	Product Conc.	Contact time	Log N_0: Start	Log N_a: End	Log Reduction (Log R= ≥ 5)	Evaluation
Enterococcus hirae ATCC 10541	Obligatory, Clean Conditions	10%	1 Minute	7.48	>3.52	<3.96	
		50%	1 Minute	7.48	>3.52	<3.96	
		Neat	1 Minute	7.48	<2.15	>5.33	Pass
Escherichia coli ATCC 10536	Obligatory, Clean Conditions	10%	1 Minute	7.51	>3.52	<3.99	
		50%	1 Minute	7.51	>3.52	<3.99	
		Neat	1 Minute	7.51	<2.15	>5.36	Pass
Pseudomonas aeruginosa ATCC 15442	Obligatory, Clean Conditions	10%	1 Minute	7.51	>3.52	<3.99	
		50%	1 Minute	7.51	>3.52	<3.99	
		Neat	1 Minute	7.51	<2.15	>5.36	Pass
Staphylococcus aureus ATCC 6538	Obligatory, Clean Conditions	10%	1 Minute	7.49	>3.52	<3.97	
		50%	1 Minute	7.49	>3.52	<3.97	
		Neat	1 Minute	7.49	<2.15	>5.34	Pass

f) Conclusions:

According to SANS 51276, the test product **Hand 'n Sani Liquid Hand Sanitizer** when used at Neat concentration achieved bactericidal activity (*logR* ≥ 5) under the following test conditions:

□ **Contact time:** 1 Minute
□ **Temperature:** 20 °C
□ **Interfering substance:** 0.3g/l Bovine Albumin, Clean Conditions
□ **Test strains**: *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541

ORGANISM: *Enterococcus hirae* ATCC 10541

Table 1a: Validation test
Obligatory Experimental Conditions

Validation suspension (Nv_0)			Experimental Conditions Control (A)= NvA			Neutralizer Control (B)= NvB			Method Validation (C) (Neat Product Concentration)= NvC		
		Ave			Ave			Ave			Ave
*Vc*1	31	30.5	*Vc*1	28	29	*Vc*1	26	27	*Vc*1	21	23
*Vc*2	30		*Vc*2	30		*Vc*2	28		*Vc*2	25	
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 = 15.25											

Table 1b: Test suspensions

Dilution (Test suspension)	*Vc*1	*Vc*2	Average *N* (wm)	Log *N*	N_0	Log N_0
10^{-6}	311	300	$3.05x10^8$	8.48	$3.05x10^7$	7.48
10^{-7}	31	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 (wm) counts: 10.02		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
10%	>330	>330	>3300	>3.52	<3.96	1 Minute
50%	>330	>330	>3300	>3.52	<3.96	1 Minute
Neat	<14	<14	<140	<2.15	>5.33	1 Minute

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* ATCC 10536

Table 2a: Validation test
Obligatory Experimental Conditions

Validation suspension (Nv_0)			Experimental Conditions Control (A)= NvA			Neutralizer Control (B)= NvB			Method Validation (C) (Neat Product Concentration)= NvC		
		Ave			Ave			Ave			Ave
*Vc*1	32	32.5	*Vc*1	30	31.5	*Vc*1	31	29	*Vc*1	29	27.5
*Vc*2	33		*Vc*2	33		*Vc*2	27		*Vc*2	26	
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 = 16.25											

Table 1b: Test suspensions

Dilution (Test suspension)	*Vc*1	*Vc*2	Average *N* (wm)	Log *N*	N_0	Log N_0
10^{-6}	322	325	$3.24x10^8$	8.51	$3.24x10^7$	7.51
10^{-7}	32	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 (wm) counts: 9.95		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: 7.51)	Contact time
10%	>330	>330	>3300	>3.52	<3.99	1 Minute
50%	>330	>330	>3300	>3.52	<3.99	1 Minute
Neat	<14	<14	<140	<2.15	>5.36	1 Minute

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* ATCC 15442

Table 3a: Validation test
Obligatory Experimental Conditions

Validation suspension (Nv_0)			Experimental Conditions Control (A)= NvA			Neutralizer Control (B)= NvB			Method Validation (C) (Neat Product Concentration)= NvC		
		Ave			Ave			Ave			Ave
*Vc*1	33	33.5	*Vc*1	30	31.5	*Vc*1	32	32	*Vc*1	24	23
*Vc*2	34		*Vc*2	33		*Vc*2	32		*Vc*2	22	
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 = 16.75											

Table 1b: Test suspensions

Dilution (Test suspension)	*Vc*1	*Vc*2	Average *N* (wm)	Log *N*	N_0	Log N_0
10^{-6}	328	320	$3.25x10^8$	8.51	$3.25x10^7$	7.51
10^{-7}	33	34				
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 (wm) counts: 9.67		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: 7.51)	Contact time
10%	>330	>330	>3300	>3.52	<3.99	1 Minute
50%	>330	>330	>3300	>3.52	<3.99	1 Minute
Neat	<14	<14	<140	<2.15	>5.36	1 Minute

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* ATCC 6538

Table 4a: Validation test
Obligatory Experimental Conditions

Validation suspension (Nv_0)			Experimental Conditions Control (A)= NvA			Neutralizer Control (B)= NvB			Method Validation (C) (Neat Product Concentration)= NvC		
		Ave			Ave			Ave			Ave
*Vc*1	31	31	*Vc*1	27	28	*Vc*1	25	25.5	*Vc*1	20	20.5
*Vc*2	31		*Vc*2	29		*Vc*2	26		*Vc*2	21	
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 = 15.5											

Table 1b: Test suspensions

Dilution (Test suspension)	*Vc*1	*Vc*2	Average *N* (wm)	Log *N*	N_0	Log N_0
10^{-6}	310	305	$3.08x10^8$	8.49	$3.08x10^7$	7.49
10^{-7}	31	31				
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 (wm) counts: 9.92		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: 7.49)	Contact time
10%	>330	>330	>3300	>3.52	<3.97	1 Minute
50%	>330	>330	>3300	>3.52	<3.97	1 Minute
Neat	<14	<14	<140	<2.15	>5.34	1 Minute

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 obligatory test conditions.
- The bactericidal concentration for a specific purpose is the concentration of the tested product for which at least a 5 log reduction is demonstrated in a *valid test* under the additional chosen test conditions. The product shall have passed under the obligatory test conditions.

Special remarks regarding results:

- All controls and validation were within the basic limits.
- At least one concentration of the product demonstrated a log reduction of less than 5 lg.
- No precipitate during the test procedure (test mixtures were homogeneous).

Notes:

Note 1: *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 an 80% solution*

Portia Nemakanga
Technical Signatory

Lungisa Qomoyi
Technical Signatory