BS EN 1276 Analysis of Naturama G3 A-5 Concentrate
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Project Report Prepared for Green Life Development Inc.

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BS EN 1276 Analysis of Naturama G3 A-5.

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Whilst these analyses have been carried out carefully and have been checked, no liabilities can be accepted for consequential or indirect damages.

Tests Carried Out By:	**Hygiene and Disinfection Centre** School of Applied Sciences University of Huddersfield Queensgate Huddersfield HD1 3DH
Test Method	British/European Standard BS EN 1276
Test Procedures	Full details of all the test and control procedures used are given in the Test Method
Disinfectant	**Naturama G3 A-5-Concentrate** Date of delivery: December 2014 Storage conditions: 20°C – 25°C
Interfering Substance	Dirty conditions 3 g l^{-1} Bovine serum albumin. Clean conditions 0.3 g l^{-1} Bovine serum albumin
Temperature	20°C
Culture Medium	Tryptone Soy- Agar, LabM
Diluent	Maximum Recovery Diluent (MRD)
Neutraliser	78g/l DE Neutralising Broth

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1 Introduction

A sample of Naturama G3 A-5-Concentrate was supplied for the following analyses:

- Bactericidal activity employing BS EN1276[1] against*, Pseudomonas aeruginosa, Escherichia coli, Enterococcus hirae, Staphylococcus aureus* under clean and dirty conditions.

1.1 Product

Product was tested undiluted.

2 Test Procedures

2.1 BS EN1276

The test was carried out as specified by BS EN1276[1] (Appendix 1). Briefly this involves the preparation of a standard suspension of test organisms containing 1.5 – 5.0 x 10^8 cells ml^{-1} of the four standard bacteria *Escherichia coli* 8879 (NCIMB); *Enterococcus hirae* 8192 (NCIMB); *Pseudomonas aeruginosa* 10421 (NCIMB) and *Staphylococcus aureus* 9518 (NCIMB).

In order to carry out the test 1 ml of interfering substance (0.3 gl^{-1} Bovine Serum Albumin (BSA) Clean conditions and 3 gl^{-1} BSA Dirty conditions) was pipetted into a Universal bottle, followed by 1 ml of the desired bacterial suspension. The mixture was mixed and left for 2 minutes at 20°C, after which 8 ml of product was added and mixed. The reaction mixture was then left for 5 minutes at 20°C, after this contact time a 1 ml sample was transferred to a tube containing 8 ml of neutraliser and 1 ml of water and left for a further 5 minutes at 20°C. The neutralisation mixture was then plated onto Tryptone Soy Agar (TSA) and incubated at 37ºC for 24 to 48 hours. Following incubation the fraction of surviving organisms was noted and a log reduction factor calculated. In addition to the test procedure outlined above a range of validations were performed to ensure the validity of the test (Appendix 1 and 2).

2.1.1 Requirements of this standard

The product, when tested as stipulated under the required test conditions (clean and dirty, 20ºC, 5 minute contact time, for the selected reference strains), shall demonstrate at least a 5 $\log_{10}$ reduction in viable counts.

2.2 Neutraliser

The neutraliser used for this test contained: 78g/l DE Neutralising Broth prepared in distilled water and autoclaved for 15 minutes at 121ºC.

3 Results and Conclusions

The product Naturama G3 A-5-Concentrate was able to generate the required >5 Log reduction in the bacteria *Pseudomonas aeruginosa, Escherichia coli, Enterococcus hirae* and *Staphylococcus aureus* within a 5 minute contact time under clean and dirty conditions.

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Organism	**Log Reduction Dirty**
S. aureus	> 5.0 Log^{rnd}
P. aeruginosa	> 4.8 Log^{rnd}
E. hirae	> 5.0 Log^{rnd}
E. coli	> 5.2 Log^{rnd}
Log^{rdn} – Log10 reduction factor	

Table 1 BS EN 1276 Dirty Conditions Results

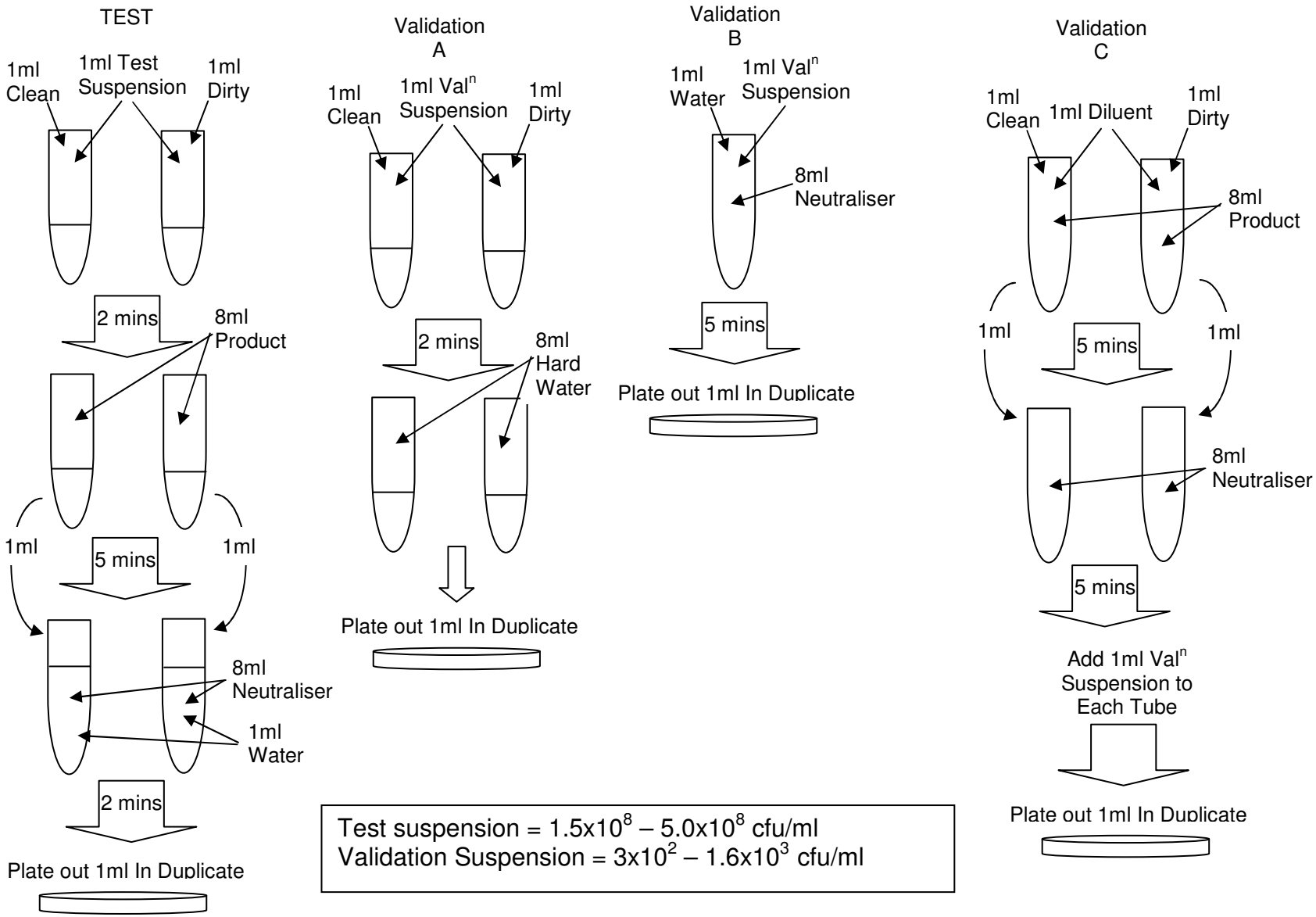
Organism	**Log Reduction Clean**
S. aureus	> 5.0 Log^{rnd}
P. aeruginosa	> 5.1 Log^{rnd}
E. hirae	> 5.0 Log^{rnd}
E. coli	> 5.2 Log^{rnd}
Log^{rdn} – Log10 reduction factor	

Table 2 BS EN 1276 Clean Conditions Results

4 References

1. BSI (2009) *BSEN 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).* British Standards Institute, London.

BSEN 1276 Flow Sheet.



Test suspension = $1.5 \times 10^8 - 5.0 \times 10^8$ cfu/ml
Validation Suspension = $3 \times 10^2 - 1.6 \times 10^3$ cfu/ml

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Appendix 2
Naturama G3 A-5-Concentrate BS EN 1276 Results

Test Organism	VALIDATIONS: Bacterial Suspension	Experimental Conditions Validation: Clean	Experimental Conditions Validation: Dirty	Neutraliser Toxicity Control	Dilution Neutralisation Control: Clean	Dilution Neutralisation Control: Dirty	Bacterial Test Suspension	Test Procedure Results: Clean	Test Procedure Results: Dirty
E. coli	53 61	Vc 43 45	44 45	Vc 28 45	Vc 28 29	28 35	10-7 284 282	Vc < 15 15	< 15 15
							10-8 24 28	Na < 1.5E+02	< 1.5E+02
	Nvo 5.7E+01	A 4.4E+01	4.5E+01	B 3.7E+01	C 2.9E+01	3.2E+01	N 2.8E+08	R > 1.9E+05	> 1.9E+05

Verification of Methodology		Passed	Log10 Reductions/cfu/ml	
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	2.8E+08	Yes	Clean	5.27247
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	5.7E+02	Yes	Dirty	5.27247
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo =	2.9E+01	Yes		
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo=	2.9E+01	Yes		
B ≥ 0.5 x Nvo when 0.5 x Nvo =	2.9E+01	Yes		
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo =	1.8E+01	Yes		
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo =	1.8E+01	Yes		

Test Organism	VALIDATIONS: Bacterial Suspension	Experimental Conditions Validation: Clean	Experimental Conditions Validation: Dirty	Neutraliser Toxicity Control	Dilution Neutralisation Control: Clean	Dilution Neutralisation Control: Dirty	Bacterial Test Suspension	Test Procedure Results: Clean	Test Procedure Results: Dirty
E.hirae	53 72	Vc 65 57	53 72	Vc 33 41	Vc 38 41	23 41	10-7 169 158	Vc < 15 15	< 15 15
							10-8 14 16	Na < 1.5E+02	< 1.5E+02
	Nvo 6.3E+01	A 6.1E+01	6.3E+01	B 3.7E+01	C 4.0E+01	3.2E+01	N 1.6E+08	R > 1.1E+05	> 1.1E+05

Verification of Methodology		Passed	Log10 Reductions/cfu/ml	
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	1.6E+08	Yes	Clean	5.03415
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	6.3E+02	Yes	Dirty	5.03415
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo =	3.1E+01	Yes		
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo=	3.1E+01	Yes		
B ≥ 0.5 x Nvo when 0.5 x Nvo =	3.1E+01	Yes		
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo =	1.9E+01	Yes		
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes		

Test Organism	VALIDATIONS: Bacterial Suspension	Experimental Conditions Validation: Clean	Experimental Conditions Validation: Dirty	Neutraliser Toxicity Control	Dilution Neutralisation Control: Clean	Dilution Neutralisation Control: Dirty	Bacterial Test Suspension	Test Procedure Results: Clean	Test Procedure Results: Dirty
P.aeruginosa	48 51	Vc 51 50	69 54	Vc 68 68	Vc 42 45	49 49	10-7 210 181	Vc < 15 15	28 29
							10-8 18 17	Na < 1.5E+02	< 2.9E+02
	Nvo 5.0E+01	A 5.1E+01	6.2E+01	B 6.8E+01	C 4.4E+01	4.9E+01	N 1.9E+08	R > 1.3E+05	> 6.8E+04

Verification of Methodology		Passed	Log10 Reductions/cfu/ml	
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	1.9E+08	Yes	Clean	5.1109
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	5.0E+02	Yes	Dirty	4.83214
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo =	2.5E+01	Yes		
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo=	2.5E+01	Yes		
B ≥ 0.5 x Nvo when 0.5 x Nvo =	2.5E+01	Yes		
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo =	3.4E+01	Yes		
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo =	3.4E+01	Yes		

Test Organism	VALIDATIONS: Bacterial Suspension	Experimental Conditions Validation: Clean	Experimental Conditions Validation: Dirty	Neutraliser Toxicity Control	Dilution Neutralisation Control: Clean	Dilution Neutralisation Control: Dirty	Bacterial Test Suspension	Test Procedure Results: Clean	Test Procedure Results: Dirty
S.aureus	32 43	Vc 33 29	41 58	Vc 49 38	Vc 32 44	35 34	10-7 152 157	Vc < 15 15	< 15 15
							10-8 15 15	Na < 1.5E+02	< 1.5E+02
	Nvo 3.8E+01	A 3.1E+01	5.0E+01	B 4.4E+01	C 3.8E+01	3.5E+01	N 1.5E+08	R > 1.0E+05	> 1.0E+05

Verification of Methodology		Passed	Log10 Reductions/cfu/ml	
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	1.5E+08	Yes	Clean	5.01169
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	3.8E+02	Yes	Dirty	5.01169
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes		
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo=	1.9E+01	Yes		
B ≥ 0.5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes		
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo =	2.2E+01	Yes		
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo =	2.2E+01	Yes		

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