Bactericidal analysis of SPRAYSAN

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Project Report Prepared for Chemanglia Ltd

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Bactericidal analysis of SPRAYSAN

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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 1276:1997. Dilution neutralisation approach
Test Procedures	Full details of all the test and control procedures used are given in the Test Method
Disinfectant	SPRAYSAN Date of delivery: January 2013 Storage conditions: 20°C – 25°C
Interfering Substance (Organic Challenge)	Simulated clean conditions: 0.3 g l^{-1} bovine albumin (final concentration) Simulated dirty conditions: 3 g l^{-1} bovine albumin (final concentration)
Temperature	20°C
Contact Time Tested	30 seconds, 1 minute, 3 minutes and 5 minutes.
Test Organisms	*Escherichia coli* 8879 (NCIMB) *Enterococus hirae* 8191 (NCIMB) *Pseudomonas aeruginosa* 10421 (NCIMB) *Staphylococus aureus* 9518 (NCIMB)
Culture Medium	Tryptone Soya Agar, LabM.
Incubation	37ºC for 24-48hrs.
Diluent	MRD, Lab M
Neutraliser	10g/L sodium thiosulphate, 12g/L saponin and 0.4g/L L-histidine.

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1 Introduction

A Sample of SPRAYSAN was submitted for the following analysis:

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

1.1 Product

The product required no dilution.

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}. The four standard bacteria; *Escherichia coli* 8879 (NCIMB); *Enterococcus hirae* 8191 (NCIMB); *Pseudomonas aeruginosa* 10421 (NCIMB) and *Staphylococcus aureus* 9518 (NCIMB) were selected for testing.

In order to carry out the test 1ml of interfering substance (0.3 gl^{-1} Bovine Serum Albumin (BSA) Clean conditions and 3.0 gl^{-1} Bovine Serum Albumin (BSA) Dirty conditions) was pipetted into a Universal bottle, followed by 1ml of the desired bacterial suspension. The mixture was vortexed and left for 2 minutes at 20°C, after which 8ml of product was added and vortexed. The reaction mixture was then left for 5 minutes at 20°C, after this contact time a 1ml sample was transferred to a tube containing 8 ml of neutraliser and 1ml of water and left for a further 5 minutes at 20°C. The neutralisation mixture was then plated onto Tryptone Soya 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). At the customers' request the test was carried out with additional contact times of 30 seconds, 1 minute and 3 minutes.

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. Additional contact times of 30 seconds, 1 minute and 3 minutes were also included at the customers' request.

2.2 Neutraliser

10g/L sodium thiosulphate, 12g/L saponin and 0.4g/L L-histidine was prepared in de-ionised water and sterilised at 121℃ for 15 minutes.

3 Results and Conclusions.

The BSEN 1276 results (Table 1, Appendix 2) show that under both clean and dirty conditions, SPRAYSAN was capable of generating the >5 Log reduction in viable counts required to pass the test against the selected organisms within 5 minutes. The additional contact times tested suggest that the >5 Log reduction in viable counts is achieved within 30 seconds.

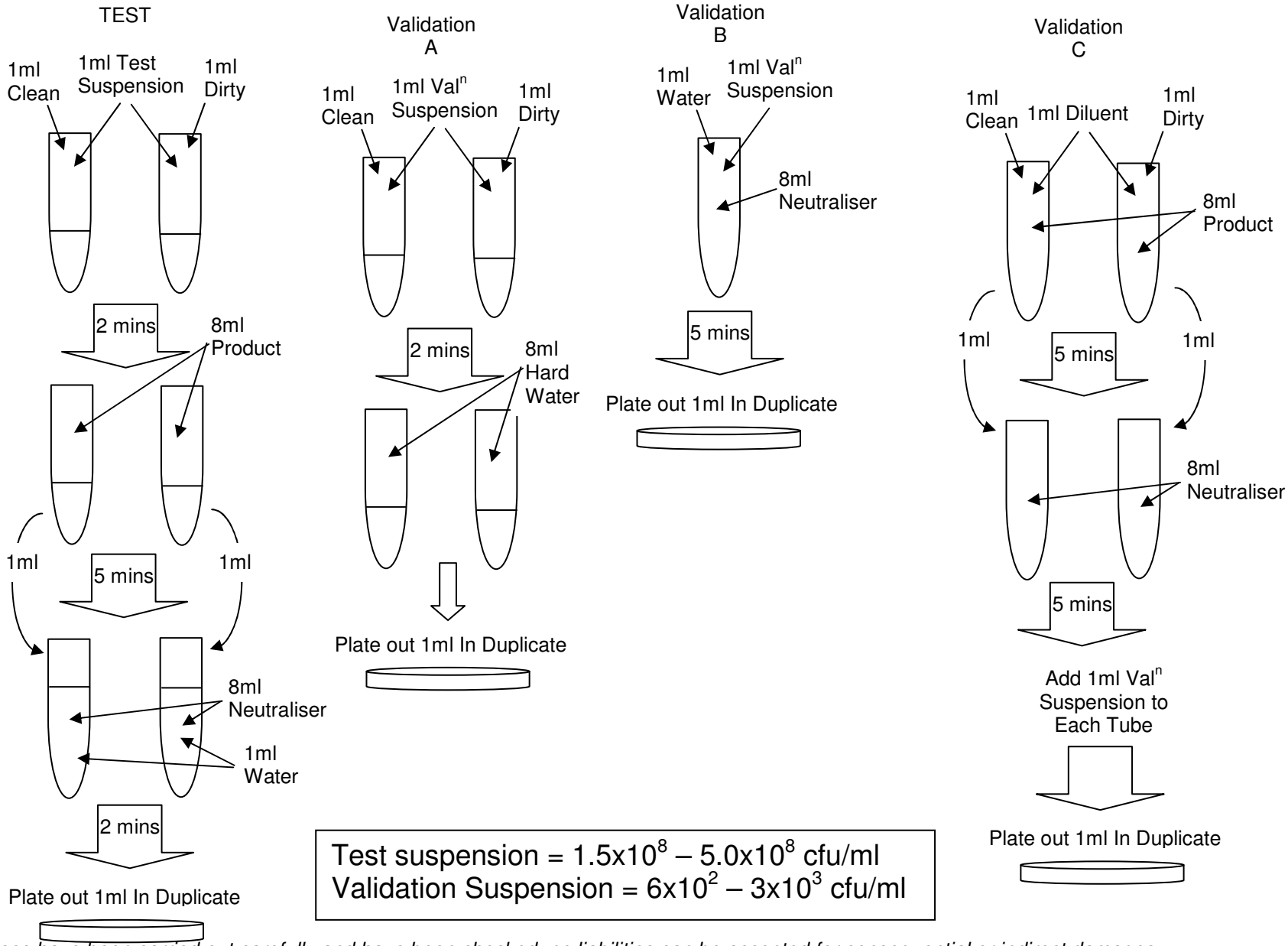
	Performance							
	E.coli		E. Hirae		P.aeruginosa		S. aureus	
Contact time	**Clean**	**Dirty**	**Clean**	**Dirty**	**Clean**	**Dirty**	**Clean**	**Dirty**
30 seconds	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}
1 minute	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}
3 minute	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}
5 minute	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}	>5.0 Log^{rdn}
Log^{rdn}-Log_{10} reduction in viable counts								

Table 1. BS EN1276 Results

4 References

1. BSI (1997) *BSEN 1276:1997. 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 = $1.5x10^8 – 5.0x10^8$ cfu/ml
Validation Suspension = $6x10^2 – 3x10^3$ cfu/ml

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Appendix 2 Results:
SPRAYSAN

Test Organism	VALIDATIONS								Bacterial Test Suspension			Test Procedure Results 30s		Test Procedure Results 1 min		Test Procedure Results 3 min		Test Procedure Results5min	
	Bacterial Suspension	Experimental Conditions			Neutraliser Toxicity Control	Dilution Neutralisation Control						Clean	Dirty	Clean	Dirty	Clean	Dirty	Clean	Dirty
		Clean		Dirty		Clean		Dirty											
E.coli		Vc 167	162	171	173	Vc 156	158	Vc 157	151	164	169	10-6 157	164	Vc < 15	15 < 15	15 Vc < 15	15 < 15	15 Vc < 15	15 < 15	15
												10-7 18	15	Na < 1.5E+02	< 1.5E+02	Na < 1.5E+02	< 1.5E+02	Na < 1.5E+02	< 1.5E+02	
	Nv 1.6E+03	A 1.6E+02		1.7E+02		B 1.6E+02		C 1.5E+02		1.7E+02		N 1.6E+08		R > 1.1E+05	> 1.1E+05	R > 1.1E+05	> 1.1E+05	R > 7.2E+01	> 7.2E+01	
	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 1min		5.0	Dirty 1min		5.0									
	Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =				1.6E+03 Yes	3min		5.0	3min		5.0									
	CLEAN A≥ 0.05 x Nv when 0.05 x Nv =				8.0E+01 Yes	5min		5.0	5min		5.0									
	DIRTY A≥ 0.05 x Nv when 0.05 x Nv =				8.0E+01 Yes															
	B≥ 0.05 x Nv when 0.05 x Nv =				8.0E+01 Yes															
	CLEAN C≥ 0.5 x B when 0.5 x B =				7.9E+01 Yes															
	DIRTY C≥ 0.5 x B when 0.5 x B =				7.9E+01 Yes															

Test Organism	VALIDATIONS								Bacterial Test Suspension			Test Procedure Results1min		Test Procedure Results 3min		Test Procedure Results5min		Test Procedure Results5min	
	Bacterial Suspension	Experimental Conditions			Neutraliser Toxicity Control	Dilution Neutralisation Control						Clean	Dirty	Clean	Dirty	Clean	Dirty	Clean	Dirty
		Clean		Dirty		Clean		Dirty											
E.hirae		Vc 162	158	175	182	Vc 171	168	Vc 173	174	187	188	10-6 196	191	Vc < 15	15 < 15	15 Vc < 15	15 < 15	15 Vc < 15	15 < 15	15
												10-7 18	19	Na < 1.5E+02	< 1.5E+02	Na < 1.5E+02	< 1.5E+02	Na < 1.5E+02	< 1.5E+02	
	Nv 1.9E+03	A 1.6E+02		1.8E+02		B 1.7E+02		C 1.7E+02		1.9E+02		N 1.9E+08		R > 1.3E+05	> 1.3E+05	R > 1.3E+05	> 1.3E+05	R > 8.6E+01	> 8.6E+01	
	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 1min		5.1	Dirty 1min		5.1									
	Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =				1.9E+03 Yes	3min		5.1	3min		5.1									
	CLEAN A≥ 0.05 x Nv when 0.05 x Nv =				9.6E+01 Yes	5min		5.1	5min		5.1									
	DIRTY A≥ 0.05 x Nv when 0.05 x Nv =				9.6E+01 Yes															
	B≥ 0.05 x Nv when 0.05 x Nv =				9.6E+01 Yes															
	CLEAN C≥ 0.5 x B when 0.5 x B =				8.5E+01 Yes															
	DIRTY C≥ 0.5 x B when 0.5 x B =				8.5E+01 Yes															

Test Organism	VALIDATIONS								Bacterial Test Suspension			Test Procedure Results 30s		Test Procedure Results 1 min		Test Procedure Results 3 min		Test Procedure Results5min	
	Bacterial Suspension	Experimental Conditions			Neutraliser Toxicity Control	Dilution Neutralisation Control						Clean	Dirty	Clean	Dirty	Clean	Dirty	Clean	Dirty
		Clean		Dirty		Clean		Dirty											
P.aeruginosa		Vc 171	166	206	185	Vc 197	220	Vc 169	166	172	168	10-6 158	186	Vc < 15	15 < 15	15 Vc < 15	15 < 15	15 Vc < 15	15 < 15	15
												10-7 19	16	Na < 1.5E+02	< 1.5E+02	Na < 1.5E+02	< 1.5E+02	Na < 1.5E+02	< 1.5E+02	
	Nv 1.7E+03	A 1.7E+02		2.0E+02		B 2.1E+02		C 1.7E+02		1.7E+02		N 1.7E+08		R > 1.1E+05	> 1.1E+05	R > 1.1E+05	> 1.1E+05	R > 7.7E+01	> 7.7E+01	
	Verification of Methodology				Passed	Log10 Reductions/cfu/ml														
	N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =				1.7E+08 Yes	Clean 1min		5.1	Dirty 1min		5.1									
	Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =				1.7E+03 Yes	3min		5.1	3min		5.1									
	CLEAN A≥ 0.05 x Nv when 0.05 x Nv =				8.6E+01 Yes	5min		5.1	5min		5.1									
	DIRTY A≥ 0.05 x Nv when 0.05 x Nv =				8.6E+01 Yes															
	B≥ 0.05 x Nv when 0.05 x Nv =				8.6E+01 Yes															
	CLEAN C≥ 0.5 x B when 0.5 x B =				1.0E+02 Yes															
	DIRTY C≥ 0.5 x B when 0.5 x B =				1.0E+02 Yes															

Test Organism	VALIDATIONS								Bacterial Test Suspension			Test Procedure Results1min		Test Procedure Results 3min		Test Procedure Results5min		Test Procedure Results5min	
	Bacterial Suspension	Experimental Conditions			Neutraliser Toxicity Control	Dilution Neutralisation Control						Clean	Dirty	Clean	Dirty	Clean	Dirty	Clean	Dirty
		Clean		Dirty		Clean		Dirty											
S.aureus		Vc 168	170	186	192	Vc 163	154	Vc 154	153	175	169	10-6 173	170	Vc < 15	15 < 15	15 Vc < 15	15 < 15	15 Vc < 15	15 < 15	15
												10-7 17	18	Na < 1.5E+02	< 1.5E+02	Na < 1.5E+02	< 1.5E+02	Na < 1.5E+02	< 1.5E+02	
	Nv 1.7E+03	A 1.7E+02		1.9E+02		B 1.6E+02		C 1.5E+02		1.7E+02		N 1.7E+08		R > 1.1E+05	> 1.1E+05	R > 1.1E+05	> 1.1E+05	R > 7.6E+01	> 7.6E+01	
	Verification of Methodology				Passed	Log10 Reductions/cfu/ml														
	N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =				1.7E+08 Yes	Clean 1min		5.1	Dirty 1min		5.1									
	Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =				1.7E+03 Yes	3min		5.1	3min		5.1									
	CLEAN A≥ 0.05 x Nv when 0.05 x Nv =				8.6E+01 Yes	5min		5.1	5min		5.1									
	DIRTY A≥ 0.05 x Nv when 0.05 x Nv =				8.6E+01 Yes															
	B≥ 0.05 x Nv when 0.05 x Nv =				8.6E+01 Yes															
	CLEAN C≥ 0.5 x B when 0.5 x B =				7.9E+01 Yes															
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