Determination of Bactericidal Activity of Zwiteck Sanitizer using the European Standard Test Method BS EN 1276:2009

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Project Report Prepared for Zwiteck BV

University of HUDDERSFIELD

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Determination of Bactericidal Activity of Zwiteck Sanitizer using the European Standard Test Method BS EN 1276:2009

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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.

Address

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Huddersfield
United Kingdom
HD1 3DH

Test Procedure BSEN 1276:2009 Dilution neutralisation approach

Test Product Zwiteck Sanitizer powder blend with dye (0.2g/100g)

Organisms

Escherichia coli 8879 (NCIMB)
Enterococcus hirae 8191 (NCIMB)
Pseudomonas aeruginosa 10421 (NCIMB)
Staphylococcus aureus 9518 (NCIMB).

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1 Introduction

A sample of Zwiteck Sanitizer powder blend with dye (0.2g/100g) was submitted the following analysis:

- Bactericidal activity employing BS EN1276[1] dirty conditions against *S.aureus* and *P.aeruginosa* to identify the lowest concentration of powder blend required to pass under these conditions
- Bactericidal activity of this lowest concentration of powder blend against all four candidate organisms in clean and dirty conditions.

1.1 Product

The 0.04g of dye was added to 20g of powder blend. This dye incorporated powder blend was diluted to concentrations of 10g/l, 5g/l, 2.5g/l, 1g/l and 0.5g/l in hard water at a temperature of 35°C.

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* 8191 (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, 3.0 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 20ºC, after which 8 ml of product was added and mixed. The reaction mixture was then left for 5 minutes at 20ºC, 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 20ºC. 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).

2.1.1 Requirements of this standard

The product, when tested as stipulated under the required test conditions (clean and dirty at 20ºC, 5 minute contact time, for the selected reference strains), shall demonstrate at least a 5 $\log_{10}$ reduction in viable counts. The test as carried out as stipulated in **1**.

2.2 Neutraliser

The neutraliser used contained: 12g/l saponin, 0.4g/l L-histidine and 10g/L sodium thiosulphate, prepared in de-ionised water and autoclaved for 15 minutes at 121ºC.

3 Results and Conclusions

The results obtained against *S.aureus* and *P.aeruginosa* in dirty conditions (Figure 1, Appendix 2) indicated that the powder blend was capable of the generating required >5.0 Log reduction in microorganisms within 5 minutes when concentration was reduced to 1g/l. When formulated at 0.5g/l, a >5.0 Log reduction in bacterial numbers was no longer achieved.

Formulation (g/L)	**Performance at 5 minutes**	
	Pseudomonas Aeruginosa	***Staphylococcus aureus***
10	>5.0 Log^{Rdn}	>5.0 Log^{Rdn}
5	>5.0 Log^{Rdn}	>5.0 Log^{Rdn}
2.5	>5.0 Log^{Rdn}	>5.0 Log^{Rdn}
1	>5.0 Log^{Rdn}	>5.0 Log^{Rdn}
0.5	<2.8 Log^{Rdn}	<2.8 Log^{Rdn}
Log^{Rdn}-Log Reduction Factor		

Figure 1: Performance of Zwiteck Sanitizer powder blend at a range of concentrations.

When expanded to a full test regimen (Figure 2, Appendix 2), the 1g/l powder blend was capable of generating the >5.0 Log reduction in bacterial numbers against all four candidate organisms in both clean and dirty conditions.

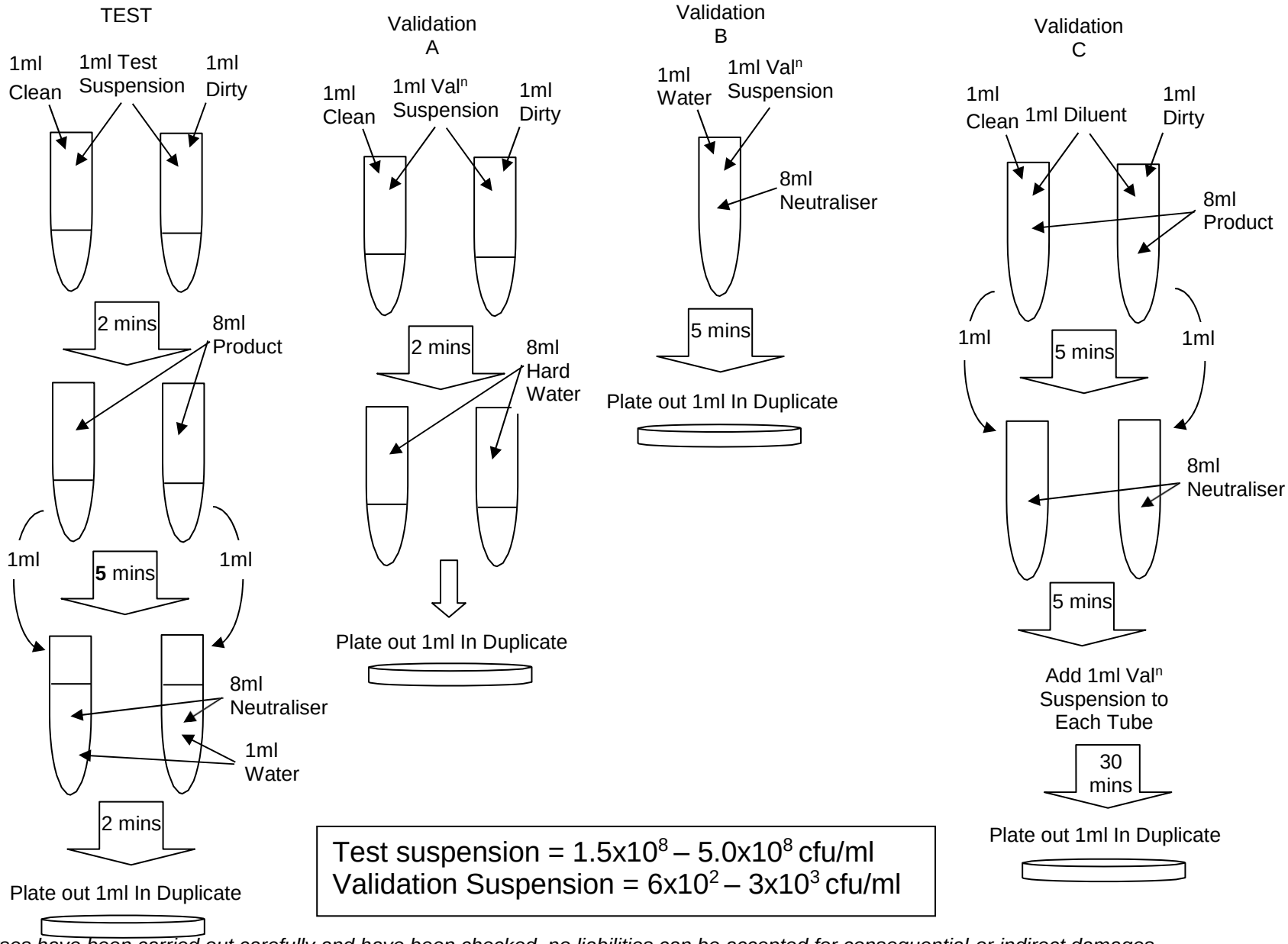
	Performance at 5 minutes	
	1g/L formulation	
Organism	**Clean conditions**	**Dirty conditions**
Enterococcus hirae	>5.0 Log^{Rdn}	>5.0 Log^{Rdn}
Escherichia coli	>5.0 Log^{Rdn}	>5.0 Log^{Rdn}
Pseudomonas Aeruginosa	>5.0 Log^{Rdn}	>5.0 Log^{Rdn}
Staphylococcus aureus	>5.0 Log^{Rdn}	>5.0 Log^{Rdn}
Log^{Rdn}-Log Reduction Factor		

Figure 2: Performance of 1g/l powder blend formulations in full BSEN 1276 test regimen.

4 References

1. BSI (2010) *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 = $6 \times 10^2 – 3 \times 10^3$ cfu/ml

Appendix 2 Results:
Minimum biocidal concentrations
Pseudomonas aeruginosa

Test Organism	VALIDATIONS						Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation		Neutraliser Toxicity Control	Dilution Neutralisation Control						
		Clean	Dirty		Clean	Dirty				Clean	Dirty
P.aeruginosa 10g/L	75 74		39 36	Vc 35 40		47 53	10-6	176 159			< 15 15
							10-7	15 20			< 1.5E+02
	Nvo 7.5E+01		3.8E+01	B 3.8E+01		5.0E+01	N	1.7E+08			> 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 = 1.7E+08 Yes				Clean #####							
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 7.5E+02 Yes				Dirty 5.05							
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+01 No											
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.7E+01 Yes											
B ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+01 Yes											
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo = 1.9E+01 No											
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo = 1.9E+01 Yes											

Test Organism	VALIDATIONS						Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation		Neutraliser Toxicity Control	Dilution Neutralisation Control						
		Clean	Dirty		Clean	Dirty				Clean	Dirty
P.aeruginosa 5g/L	75 74		39 36	Vc 35 40		33 38	10-6	176 159			< 15 15
							10-7	15 20			< 1.5E+02
	Nvo 7.5E+01		3.8E+01	B 3.8E+01		3.6E+01	N	1.7E+08			> 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 = 1.7E+08 Yes				Clean #####							
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 7.5E+02 Yes				Dirty 5.05							
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+01 No											
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.7E+01 Yes											
B ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+01 Yes											
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo = 1.9E+01 No											
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo = 1.9E+01 Yes											

Test Organism	VALIDATIONS						Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation		Neutraliser Toxicity Control	Dilution Neutralisation Control						
		Clean	Dirty		Clean	Dirty				Clean	Dirty
P.aeruginosa 2.5g/L	75 74		39 36	Vc 35 40		44 38	10-6	176 159			< 15 15
							10-7	15 20			< 1.5E+02
	Nvo 7.5E+01		3.8E+01	B 3.8E+01		4.1E+01	N	1.7E+08			> 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 = 1.7E+08 Yes											
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 7.5E+02 Yes				Dirty 5.05							
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+01 No											
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.7E+01 Yes											
B ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+01 Yes											
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo = 1.9E+01 No											
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo = 1.9E+01 Yes											

Test Organism	VALIDATIONS						Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation		Neutraliser Toxicity Control	Dilution Neutralisation Control						
		Clean	Dirty		Clean	Dirty				Clean	Dirty
P.aeruginosa 1g/L	75 74	Vc	39 36	Vc 35 40	Vc	44 38	10-6	216 212			< 15 15
							10-7	21 22			< 1.5E+02
	Nvo 7.5E+01	A	3.8E+01	B 3.8E+01	C	4.1E+01	N	2.1E+08			> 1.4E+05
Verification of Methodology Passed				Log10 Reductions/cfu/ml							
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 2.1E+08 Yes											
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 7.5E+02 Yes				Dirty 5.15							
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+01 No											
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.7E+01 Yes											
B ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+01 Yes											
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo = 1.9E+01 No											
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo = 1.9E+01 Yes											

Test Organism	VALIDATIONS						Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation		Neutraliser Toxicity Control	Dilution Neutralisation Control						
		Clean	Dirty		Clean	Dirty				Clean	Dirty
P.aeruginosa 0.5g/L	74 52	Vc	32 36	Vc 49 52	Vc	38 46	10-6	194 172		Vc	> 3000 3000
							10-7	19 17		Na	< 3.0E+04
	Nvo 6.3E+01	A	3.4E+01	B 5.1E+01	C	4.2E+01	N	1.8E+08		R	> 6.1E+02
Verification of Methodology Passed				Log10 Reductions/cfu/ml							
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 1.8E+08 Yes											
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 6.3E+02 Yes				Dirty 2.78							
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo = 3.2E+01 No											
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.2E+01 Yes											
B ≥ 0.5 x Nvo when 0.5 x Nvo = 3.2E+01 Yes											
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo = 2.5E+01 No											
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo = 2.5E+01 Yes											

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Staphylococcus aureus

Test Organism	VALIDATIONS												Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control									
		Clean		Dirty				Clean		Dirty						Clean	Dirty
S.aureus 10g/L	60 82	Vc		38 40	Vc	33	41	Vc		32 30			10-6	178	172	Vc	< 15 15
													10-7	19	17	Na	< 1.5E+02
	Nvo 7.1E+01	A		3.9E+01	B	3.7E+01		C		3.1E+01			N	1.8E+08		R	> 1.2E+05

Verification of Methodology		Passed	Log10 Reductions/cfu/ml	
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	1.8E+08	Yes		
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	7.1E+02	Yes	Dirty	5.07
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	No		
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo=	3.6E+01	Yes		
B ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	Yes		
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo =	1.9E+01	No		
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes		

Test Organism	VALIDATIONS												Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control									
		Clean		Dirty				Clean		Dirty						Clean	Dirty
S.aureus 5g/L	60 82	Vc		38 40	Vc	33	41	Vc		33 42			10-6	178	172	Vc	< 15 15
													10-7	19	17	Na	< 1.5E+02
	Nvo 7.1E+01	A		3.9E+01	B	3.7E+01		C		3.8E+01			N	1.8E+08		R	> 1.2E+05

Verification of Methodology		Passed	Log10 Reductions/cfu/ml	
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	1.8E+08	Yes		
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	7.1E+02	Yes	Dirty	5.07
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	No		
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo=	3.6E+01	Yes		
B ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	Yes		
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo =	1.9E+01	No		
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes		

Test Organism	VALIDATIONS												Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control									
		Clean		Dirty				Clean		Dirty						Clean	Dirty
S.aureus 2.5g/L	60 82	Vc		38 40	Vc	33	41	Vc		45 31			10-6	178	172	Vc	< 15 15
													10-7	19	17	Na	< 1.5E+02
	Nvo 7.1E+01	A		3.9E+01	B	3.7E+01		C		3.8E+01			N	1.8E+08		R	> 1.2E+05

Verification of Methodology		Passed	Log10 Reductions/cfu/ml	
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	1.8E+08	Yes		
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	7.1E+02	Yes	Dirty	5.068073
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	No		
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo=	3.6E+01	Yes		
B ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	Yes		
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo =	1.9E+01	No		
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes		

Test Organism	VALIDATIONS												Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control									
		Clean		Dirty				Clean		Dirty						Clean	Dirty
S.aureus 1g/L	60 82	Vc		41 56	Vc	33	41	Vc		39 42			10-6	178	172	Vc	< 15 15
													10-7	19	17	Na	< 1.5E+02
	Nvo 7.1E+01	A		4.9E+01	B	3.7E+01		C		4.1E+01			N	1.8E+08		R	> 1.2E+05

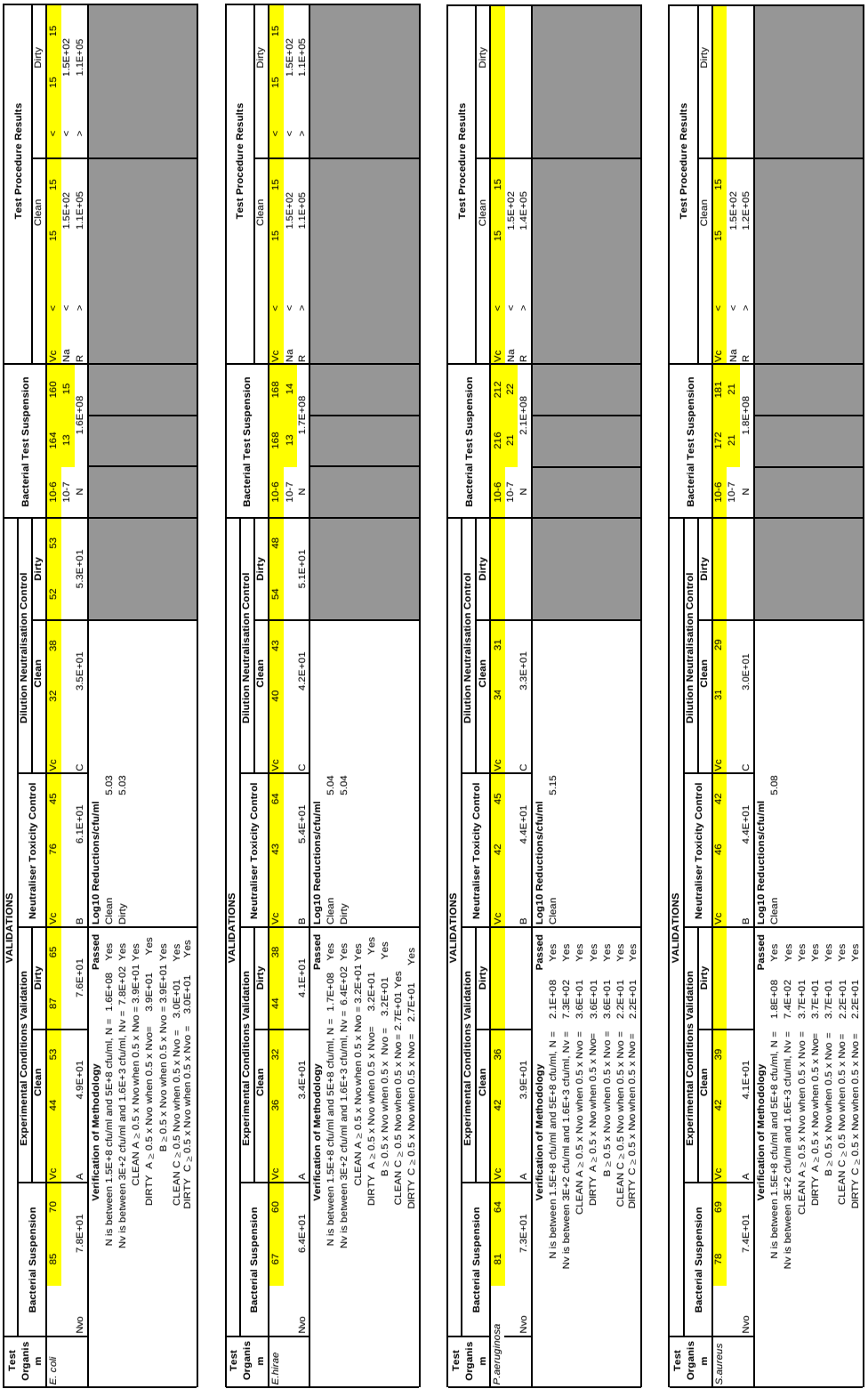
Verification of Methodology		Passed	Log10 Reductions/cfu/ml	
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	1.8E+08	Yes		
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	7.1E+02	Yes	Dirty	5.07
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	No		
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo=	3.6E+01	Yes		
B ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	Yes		
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo =	1.9E+01	No		
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes		

Test Organism	VALIDATIONS												Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control									
		Clean		Dirty				Clean		Dirty						Clean	Dirty
S.aureus 0.5g/L	62 81	Vc		46 52	Vc	49	54	Vc		40 41			10-6	204	211	Vc	> 3000 3000
													10-7	18	16	Na	< 3.0E+04
	Nvo 7.2E+01	A		4.9E+01	B	5.2E+01		C		4.1E+01			N	2.0E+08		R	> 6.8E+02

Verification of Methodology		Passed	Log10 Reductions/cfu/ml	
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	2.0E+08	Yes	Clean	
Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =	7.2E+02	Yes	Dirty	2.83
CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	No		
DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo=	3.6E+01	Yes		
B ≥ 0.5 x Nvo when 0.5 x Nvo =	3.6E+01	Yes		
CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo =	2.6E+01	No		
DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo =	2.6E+01	Yes		

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

1g/l Powder blend 1276.



VALIDATIONS

Test Organism	Bacterial Suspension	Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control			Bacterial Test Suspension			Test Procedure Results			
		Clean		Dirty				Clean		Dirty				Clean		Dirty	
E. coli	85	70	Vc	44	53	87	65	Vc	76	45	Vc	32	38	52	53	10-6	164	160	Vc	<	15	15	<	15	15
																10-7	13	15	Na	<	1.5E+02		<	1.5E+02	
	Nvo	7.8E+01	A	4.9E+01		7.6E+01		B	6.1E+01		C	3.5E+01		5.3E+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		5.03															
	Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 7.8E+02 Yes							Dirty		5.03															
	CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo = 3.9E+01 Yes																								
	DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.9E+01 Yes																								
	B ≥ 0.5 x Nvo when 0.5 x Nvo = 3.9E+01 Yes																								
	CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo = 3.0E+01 Yes																								
	DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo = 3.0E+01 Yes																								

VALIDATIONS

Test Organism	Bacterial Suspension	Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control			Bacterial Test Suspension			Test Procedure Results			
		Clean		Dirty				Clean		Dirty				Clean		Dirty	
E.hirae	67	60	Vc	36	32	44	38	Vc	43	64	Vc	40	43	54	48	10-6	168	168	Vc	<	15	15	<	15	15
																10-7	13	14	Na	<	1.5E+02		<	1.5E+02	
	Nvo	6.4E+01	A	3.4E+01		4.1E+01		B	5.4E+01		C	4.2E+01		5.1E+01		N	1.7E+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 = 1.7E+08 Yes							Clean		5.04															
	Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 6.4E+02 Yes							Dirty		5.04															
	CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo = 3.2E+01 Yes																								
	DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.2E+01 Yes																								
	B ≥ 0.5 x Nvo when 0.5 x Nvo = 3.2E+01 Yes																								
	CLEAN C ≥ 0.5 Nvo when 0.5 x Nvo = 2.7E+01 Yes																								
	DIRTY C ≥ 0.5 x Nvo when 0.5 x Nvo= 2.7E+01 Yes																								

VALIDATIONS

Test Organism	Bacterial Suspension	Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control			Bacterial Test Suspension			Test Procedure Results			
		Clean		Dirty				Clean		Dirty				Clean		Dirty	
P.aeruginosa	81	64	Vc	42	36			Vc	42	45	Vc	34	31			10-6	216	212	Vc	<	15	15			
																10-7	21	22	Na	<	1.5E+02				
	Nvo	7.3E+01	A	3.9E+01				B	4.4E+01		C	3.3E+01				N	2.1E+08		R	>	1.4E+05				
	Verification of Methodology						Passed	Log10 Reductions/cfu/ml																	
	N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 2.1E+08 Yes							Clean		5.15															
	Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 7.3E+02 Yes																								
	CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo = 3.6E+01 Yes																								
	DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.6E+01 Yes																								
	B ≥ 0.5 x Nvo when 0.5 x Nvo = 3.6E+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																								

VALIDATIONS

Test Organism	Bacterial Suspension	Experimental Conditions Validation			Neutraliser Toxicity Control			Dilution Neutralisation Control			Bacterial Test Suspension			Test Procedure Results			
		Clean		Dirty				Clean		Dirty				Clean		Dirty	
S.aureus	78	69	Vc	42	39			Vc	46	42	Vc	31	29			10-6	172	181	Vc	<	15	15			
																10-7	21	21	Na	<	1.5E+02				
	Nvo	7.4E+01	A	4.1E+01				B	4.4E+01		C	3.0E+01				N	1.8E+08		R	>	1.2E+05				
	Verification of Methodology						Passed	Log10 Reductions/cfu/ml																	
	N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 1.8E+08 Yes							Clean		5.08															
	Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 7.4E+02 Yes																								
	CLEAN A ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+01 Yes																								
	DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.7E+01 Yes																								
	B ≥ 0.5 x Nvo when 0.5 x Nvo = 3.7E+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																								

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