

**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**



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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.

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Address	Hygiene and Disinfection Centre University of Huddersfield Queensgate Campus 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	<i>Escherichia coli</i> 8879 (NCIMB) <i>Enterococcus hirae</i> 8191 (NCIMB) <i>Pseudomonas aeruginosa</i> 10421 (NCIMB) <i>Staphylococcus aureus</i> 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 EN1276¹ 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 EN1276¹ (Appendix 1). Briefly this involves the preparation of a standard suspension of test organisms containing $1.5 - 5.0 \times 10^8$ 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 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 Soya Agar (TSA) and incubated at 37°C 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 20°C, 5 minute contact time, for the selected reference strains), shall demonstrate at least a 5 log₁₀ reduction in viable counts. The test as carried out as stipulated in 1.

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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 121°C.

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	
	<i>Pseudomonas Aeruginosa</i>	<i>Staphylococcus aureus</i>
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.

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

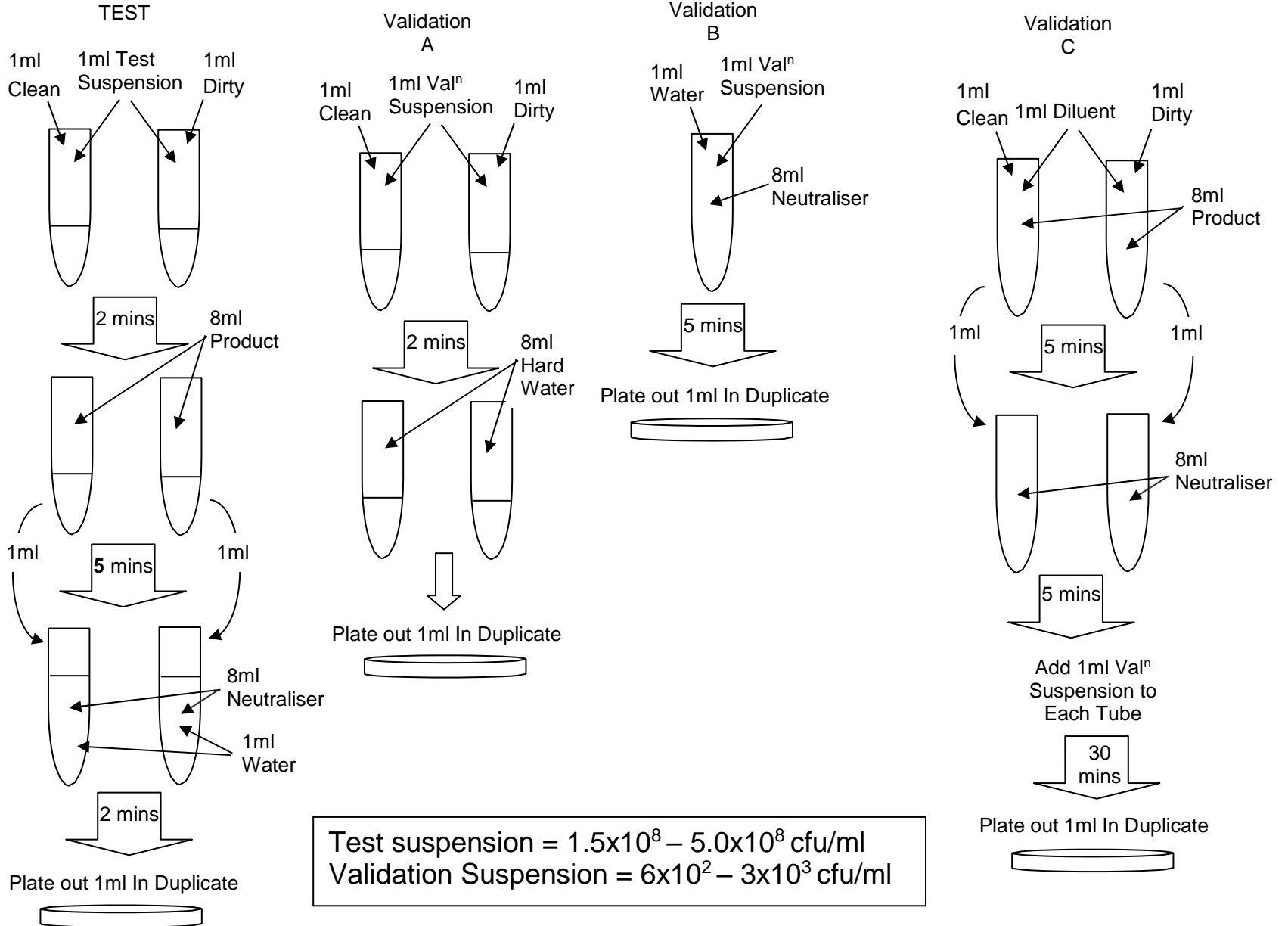
4 References

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.

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**BSEN 1276
Flow Sheet.**

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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	Clean	Dirty				
<i>P.aeruginosa</i> 10g/L	75 74		39 36	Vc 35 40		47 53	10-6 176 159		<	15 15		
	Nvo 7.5E+01		3.8E+01	B 3.8E+01		5.0E+01	10-7 15 20		<	1.5E+02		
							N 1.7E+08		>	1.1E+05		
Verification of Methodology Passed 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 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						Log10 Reductions/cfu/ml Clean ##### Dirty 5.05						

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	Clean	Dirty				
<i>P.aeruginosa</i> 5g/L	75 74		39 36	Vc 35 40		33 38	10-6 176 159		<	15 15		
	Nvo 7.5E+01		3.8E+01	B 3.8E+01		3.6E+01	10-7 15 20		<	1.5E+02		
							N 1.7E+08		>	1.1E+05		
Verification of Methodology Passed 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 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						Log10 Reductions/cfu/ml Clean ##### Dirty 5.05						

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	Clean	Dirty				
<i>P.aeruginosa</i> 2.5g/L	75 74		39 36	Vc 35 40		44 38	10-6 176 159		<	15 15		
	Nvo 7.5E+01		3.8E+01	B 3.8E+01		4.1E+01	10-7 15 20		<	1.5E+02		
							N 1.7E+08		>	1.1E+05		
Verification of Methodology Passed 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 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						Log10 Reductions/cfu/ml Dirty 5.05						

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	Clean	Dirty				
<i>P.aeruginosa</i> 1g/L	75 74	Vc	39 36	Vc 35 40	Vc	44 38	10-6 216 212		<	15 15		
	Nvo 7.5E+01	A	3.8E+01	B 3.8E+01	C	4.1E+01	10-7 21 22		<	1.5E+02		
							N 2.1E+08		>	1.4E+05		
Verification of Methodology Passed 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 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						Log10 Reductions/cfu/ml Dirty 5.15						

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	Clean	Dirty				
<i>P.aeruginosa</i> 0.5g/L	74 52	Vc	32 36	Vc 49 52	Vc	38 46	10-6 194 172	Vc	>	3000 3000		
	Nvo 6.3E+01	A	3.4E+01	B 5.1E+01	C	4.2E+01	10-7 19 17	Na	<	3.0E+04		
							N 1.8E+08	R	>	6.1E+02		
Verification of Methodology Passed 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 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						Log10 Reductions/cfu/ml Dirty 2.78						

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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	40		Clean	Dirty										
<i>S.aureus</i> 10g/L	60 82	Vc	38	40	Vc	33	41	Vc	32	30	10-6	178	172	Vc	<	15	15
	Nvo 7.1E+01	A	3.9E+01		B	3.7E+01		C	3.1E+01		10-7	19	17	Na	<	1.5E+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	Dirty			5.07								
	Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =			7.1E+02	Yes												
	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	40		Clean	Dirty										
<i>S.aureus</i> 5g/L	60 82	Vc	38	40	Vc	33	41	Vc	33	42	10-6	178	172	Vc	<	15	15
	Nvo 7.1E+01	A	3.9E+01		B	3.7E+01		C	3.8E+01		10-7	19	17	Na	<	1.5E+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	Dirty			5.07								
	Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =			7.1E+02	Yes												
	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	40		Clean	Dirty										
<i>S.aureus</i> 2.5g/L	60 82	Vc	41	56	Vc	33	41	Vc	45	31	10-6	178	172	Vc	<	15	15
	Nvo 7.1E+01	A	4.9E+01		B	3.7E+01		C	3.8E+01		10-7	19	17	Na	<	1.5E+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	Dirty			5.068073								
	Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =			7.1E+02	Yes												
	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	40		Clean	Dirty										
<i>S.aureus</i> 1g/L	60 82	Vc	41	56	Vc	33	41	Vc	39	42	10-6	178	172	Vc	<	15	15
	Nvo 7.1E+01	A	4.9E+01		B	3.7E+01		C	4.1E+01		10-7	19	17	Na	<	1.5E+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	Dirty			5.07								
	Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =			7.1E+02	Yes												
	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	52		Clean	Dirty										
<i>S.aureus</i> 0.5g/L	62 81	Vc	46	52	Vc	49	54	Vc	40	41	10-6	204	211	Vc	>	3000	3000
	Nvo 7.2E+01	A	4.9E+01		B	5.2E+01		C	4.1E+01		10-7	18	16	Na	<	3.0E+04	
	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			2.83								
	Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv =			7.2E+02	Yes												
	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												

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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															
<i>E. coli</i>	85	70	Vc	44	53	87	65	Vc	76	45	Vc	32	38	52	53	10-6	164	160	Vc	<	15	15	<	15	15		
	Nvo	7.8E+01	A	4.9E+01		7.6E+01		B	6.1E+01		C	3.5E+01		5.3E+01		10-7	13	15	Na	<	1.5E+02		<	1.5E+02			
				Verification of Methodology N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 1.6E+08 Yes Nv is between 3E+2 cfu/ml and 1.6E+3 cfu/ml, Nv = 7.8E+02 Yes 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				Passed Log10 Reductions/cfu/ml Clean 5.03 Dirty 5.03																			

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															
<i>E. hirae</i>	67	60	Vc	36	32	44	38	Vc	43	64	Vc	40	43	54	48	10-6	168	168	Vc	<	15	15	<	15	15		
	Nvo	6.4E+01	A	3.4E+01		4.1E+01		B	5.4E+01		C	4.2E+01		5.1E+01		10-7	13	14	Na	<	1.5E+02		<	1.5E+02			
				Verification of Methodology 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 = 6.4E+02 Yes 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				Passed Log10 Reductions/cfu/ml Clean 5.04 Dirty 5.04																			

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														
<i>P. aeruginosa</i>	81	64	Vc	42	36			Vc	42	45	Vc	34	31			10-6	216	212	Vc	<	15	15				
	Nvo	7.3E+01	A	3.9E+01				B	4.4E+01		C	3.3E+01				10-7	21	22	Na	<	1.5E+02					
				Verification of Methodology 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 = 7.3E+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				Passed Log10 Reductions/cfu/ml Clean 5.15																		

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															
<i>S. aureus</i>	78	69	Vc	42	39			Vc	46	42	Vc	31	29			10-6	172	181	Vc	<	15	15					
	Nvo	7.4E+01	A	4.1E+01				B	4.4E+01		C	3.0E+01				10-7	21	21	Na	<	1.5E+02						
				Verification of Methodology 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 = 7.4E+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				Passed Log10 Reductions/cfu/ml Clean 5.08																			

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