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

Project Report Prepared for Zwiteck BV



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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).

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 x 10³ 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**.

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

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.

- 14 (1)	Performance	e at 5 minutes										
Formulation (g/L)	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	Log ^{Rdn} -Log Reduction Factor											

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

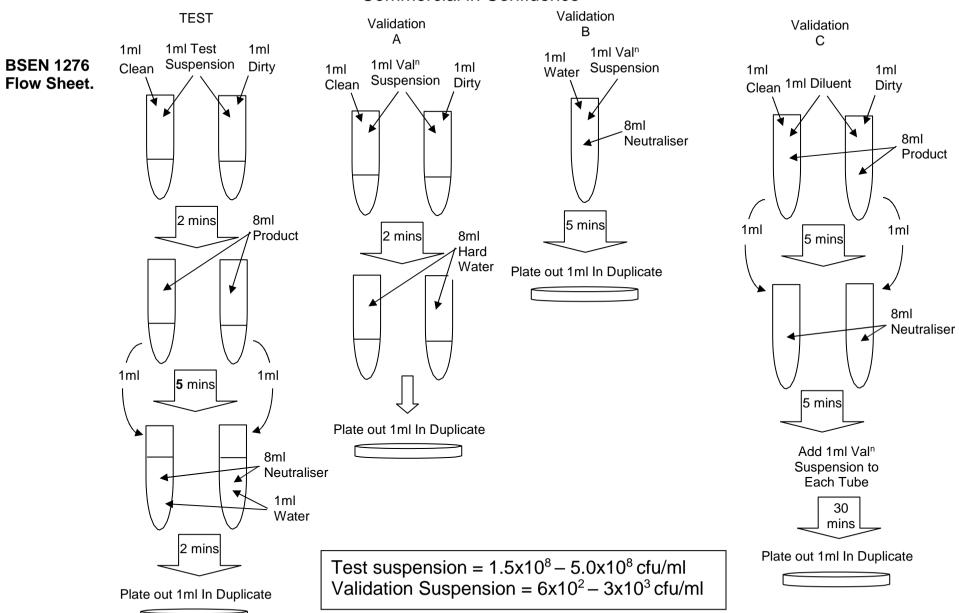
When expanded to a full test regimen (Figure 2, Appendix 2), the 1g/l powder blend was capable of generating the >5.0 Log reduction in bacterial numbers against all four candidate organisms in both clean and dirty conditions.

	Performance 1g/L forr										
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.



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

Appendix 2 Results: Minimum biocidal concentrations

Pseudomonas aeruginosa

Test			VALIDA	TIONS			Bacterial Test	Toot D	ocedure Results
Organism	Bacterial	Experimental Cond	itions Validation	Neutraliser	Dilution Neutrali	sation Control	Suspension	162111	ocedure Results
Organism	Suspension	Clean	Dirty	Toxicity Control	Clean	Dirty	Suspension	Clean	Dirty
P.aeruginosa	75 74		39 36	Vc 35 40		47 53	10-6 176 159		< 15 15
10g/L							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
	Veri	fication of Methodole	ogy Passed	Log10 Reductions	s/cfu/ml				
N is betw	een 1.5E+8 cfu/	ml and 5E+8 cfu/ml, N	= 1.7E+08 Yes	Clean ####					
Nv is bet	tween 3E+2 cfu/	ml and 1.6E+3 cfu/ml,	Nv = 7.5E+02 Yes	Dirty 5.05					
	CLEAN A 2	0.5 x Nvo when 0.5 x	Nvo = 3.7E+01 No						
1	DIRTY A≥0.5x	Nvo when 0.5 x Nvo:	= 3.7E+01 Yes						
	B ≥ 0.5 x	Nvo when 0.5 x Nv	o = 3.7E+01 Yes						
C	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			VALIDA	TIONS			Bacterial Test	Toot D	ocedure Results
Organism	Bacterial	Experimental Cond	itions Validation	Neutraliser	Dilution Neutrali	sation Control	Suspension	1621 FI	ocedure Results
Organism	Suspension	Clean	Dirty	Toxicity Control	Clean	Dirty	Suspension	Clean	Dirty
P.aeruginosa	75 74		39 36	Vc 35 40		33 38	10-6 176 159		< 15 15
5g/L							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
Nv is bet	een 1.5E+8 cfu/ tween 3E+2 cfu/ CLEAN A ≥ DIRTY A ≥ 0.5 x B ≥ 0 CLEAN C ≥ 0.5	fication of Methodoli ml and 5E+8 cfu/ml, N ml and 1.6E+3 cfu/ml, c 0.5 x Nvo when 0.5 x k Nvo when 0.5 x Nvo 0.5 x Nvo when 0.5 x Nvo Nvo when 0.5 x Nvo Nvo when 0.5 x Nvo Nvo when 0.5 x Nvo	= 1.7E+08 Yes Nv = 7.5E+02 Yes Nvo = 3.7E+01 No = 3.7E+01 Yes Nvo = 3.7E+01 Yes = 1.9E+01 No	Log10 Reductions Clean #### Dirty 5.05					

Test			VALIDA	NOITA	IS				Pag	terial Test	To	ot Broom	dure Resul	lto
Organism	Bacterial	Experimental Cond	itions Validation		Neutraliser	Dilution Neutrali	sation C	ontrol		spension	16	SI FIOCE	iure Resui	its
Organism	Suspension	Clean	Dirty	To	xicity Control	Clean	D	irty	Su	spension	Clea	n	Dir	rty
P.aeruginosa	75 74		39 36	Vc	35 40		44	38	10-6	176 159		<	15	15
2.5g/L									10-7	15 20		<	1.5E	+02
	Nvo 7.5E+01		3.8E+01	В	3.8E+01		4.1	E+01	N	1.7E+08		>	1.1E	+05
	Veri	fication of Methodole	ogy Passed	Log	g10 Reductions	s/cfu/ml								
N is between	een 1.5E+8 cfu/	ml and 5E+8 cfu/ml, N	= 1.7E+08 Yes											
Nv is bet		ml and 1.6E+3 cfu/ml,		Dirt	y 5.05									
	CLEAN A 2	0.5 x Nvo when 0.5 x	Nvo = 3.7E+01 No											
	DIRTY $A \ge 0.5 \times$	Nvo when 0.5 x Nvo:	= 3.7E+01 Yes											
	B ≥ 0.5 x	Nvo when 0.5 x Nv	ro = 3.7E + 01 Yes											
C	LEAN $C \ge 0.5$	Nvo when 0.5 x Nvo	= 1.9E+01 No											
D	IRTY C > 0.5 x	Nvo when 0.5 x Nvo =	1.9E+01 Yes											

Test			VALIDA	TIONS	3			Dan	terial '	T4	Took D		re Results
Organism	Bacterial	Experimental Cond	itions Validation	Ne	eutraliser	Dilution Neutrali	sation Control		teriai spens		rest Pi	ocea	ire Results
Organism	Suspension	Clean	Dirty	Toxi	city Control	Clean	Dirty	Su	spens	ion	Clean		Dirty
P.aeruginosa	75 74	Vc	39 36	Vc	35 40	Vc	44 38	10-6	216			<	15 15
1g/L								10-7	21	22		<	1.5E+02
	Nvo 7.5E+01	A	3.8E+01	В	3.8E+01	С	4.1E+01	N	2.1	E+08		>	1.4E+05
	Veri	ification of Methodol	ogy Passed	Log1	0 Reduction	s/cfu/ml							
N is betw	een 1.5E+8 cfu/	ml and 5E+8 cfu/ml, N	= 2.1E+08 Yes										
Nv is be	tween 3E+2 cfu/	ml and 1.6E+3 cfu/ml,	Nv = 7.5E+02 Yes	Dirty	5.15								
	CLEAN A 2	≥ 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 Nv	o = 3.7E+01 Yes										
	CLEAN C ≥ 0.5	Nvo when 0.5 x Nvo	= 1.9E+01 No										
	DIRTY $C \ge 0.5 x$	Nvo when 0.5 x Nvo =	1.9E+01 Yes										

Test			VALIDA	TIONS	3			Pag	terial T	not.	Toot D	roood	ure Results	
Organism	Bacterial	Experimental Cond	itions Validation	Ne	eutraliser	Dilution Neutrali	sation Control		spensio		Test P	rocea	ure Results	
Organism	Suspension	Clean	Dirty	Toxi	city Control	Clean	Dirty	Ju	spensio	J II	Clean		Dirty	
P.aeruginosa	74 52	Vc	32 36	Vc	49 52	Vc	38 46	10-6	194 1	72	Vc	>	3000 300	00
0.5g/L								10-7	19	17	Na	<	3.0E+04	
	Nvo 6.3E+01	A	3.4E+01	В	5.1E+01	С	4.2E+01	N	1.8E	+08	R	>	6.1E+02	
	Veri	fication of Methodolo	ogy Passed	Log1	0 Reduction	s/cfu/ml								
N is betw	een 1.5E+8 cfu/	ml and 5E+8 cfu/ml, N	= 1.8E+08 Yes											
Nv is bet	tween 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×	Nvo when 0.5 x Nvo:	= 3.2E+01 Yes											
	B ≥ 0.5 x	Nvo when 0.5 x Nv	o = 3.2E+01 Yes											
	CLEAN C ≥ 0.5	Nvo when 0.5 x Nvo	= 2.5E+01 No											
	DIRTY $C \ge 0.5 x$	Nvo when 0.5 x Nvo =	2.5E+01 Yes											

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

Staphylococcus aureus

				VALI	OATION	IS										Test Proce	d	D la -	
Test Organism	Bacterial	Experimental Condit	ions Valida	tion	Neu	traliser 1	Toxicity		Dilution Neutral	isation C	ontrol	Bacteri	al Test Sus	spension		Test Proce	aure i	Results	
	Suspension	Clean	Dir	ty	1	Contro	ol		Clean	D	irty					Clean		Di	irty
S.aureus	60 82	Vc	38	40	Vc	33	41	Vc		32	30	10-6	178	172	Vc		<	15	15
10g/L												10-7	19	17	Na		<	1.5	E+02
	Nvo 7.1E+01	A	3.9E	+01	В	3.7E	+01	С		3.1	E+01	N	1.8E	+08	R		>	1.2	E+05
	Ve	rification of Methodology		Passed	Log10	Reduction	ons/cfu/m	nl											
N is	between 1.5E+8 cf	u/ml and 5E+8 cfu/ml, N =	1.8E+08	Yes															
Nv is b	etween 3E+2 cfu/n	nl and 1.6E+3 cfu/ml, Nv =	7.1E+02	Yes	Dirty		5.07	7											
	CLEAN A \geq 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 $C \ge 0$.	5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes															

				VALIE	DATIONS										Test Proce	alessa I	D 14 -
Test Organism	Bacterial	Experimental Condit	ons Valida	tion	Neutra	aliser Toxicity	,	Dilution Neutra	isation Co	ntrol	Bacteria	al Test Sus	pension		1621 FIOCE	uure	Kesuits
	Suspension	Clean	Dirt	у		Control		Clean	Di	rty					Clean		Dirty
S.aureus	60 82	Vc	38	40	Vc	33 41	٧	/c	33	42	10-6	178	172	Vc		<	15 15
5g/L											10-7	19	17	Na		<	1.5E+02
	Nvo 7.1E+01	A	3.9E+	-01	В	3.7E+01	C		3.8E	+01	N	1.8E	+08	R		>	1.2E+05
	Ver	ification of Methodology		Passed	Log10 R	eductions/cfu	ı/ml										
N is	between 1.5E+8 cf	u/ml and 5E+8 cfu/ml, N =	1.8E+08	Yes													
Nv is b	etween 3E+2 cfu/m	nl and 1.6E+3 cfu/ml, Nv =	7.1E+02	Yes	Dirty	5	.07										
	CLEAN A \geq 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													
		0.5 Nvo when 0.5 x Nvo =		No													
	DIRTY $C \ge 0$.	5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes													

					DATION	IS											Test Proc	oduro	Dogulac	
Test Organism	Bacterial	Experimental Condit	ons Valida	tion	Neu	traliser	Toxicity		Dilution Neutral	isation C	ontro	_	Bacteria	I Test Sus	pension		Test Floc	euure	Results	
	Suspension	Clean	Dir	ty	1	Cont	rol		Clean		Dirty						Clean			Dirty
S.aureus	60 82	Vc	38	40	Vc	33	41	Vc		45		31	10-6	178	172	Vc		<	15	15
2.5g/L													10-7	19	17	Na		<		5E+02
	Nvo 7.1E+01	A	3.9E	+01	В	3.7	E+01	С		3.8	8E+01		N	1.8E	+08	R		>	1.3	2E+05
	Ve	rification of Methodology		Passed	Log10	Reduct	tions/cfu/m	ıl												
N is	between 1.5E+8 cf	u/ml and 5E+8 cfu/ml, N =	1.8E+08	Yes																
Nv is b	etween 3E+2 cfu/n	nl and 1.6E+3 cfu/ml, Nv =	7.1E+02	Yes	Dirty		5.068073	3												
	CLEAN A \geq 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	l															
	DIRTY $C \ge 0$.	5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes																

					DATIONS	3									Test Proc	adura	Beaulte
Test Organism	Bacterial	Experimental Condit	ions Valida	tion	Neut	raliser Toxic	city	Dilution Neutra	lisation Co	ntrol	Bacteria	al Test Sus	spension		1621 FIOC	euure	Results
	Suspension	Clean	Dir	ty		Control		Clean	Di	rty					Clean		Dirty
S.aureus	60 82	Vc	41	56	Vc	33	41	Vc	39	42	10-6	178	172	Vc		<	15 15
1g/L											10-7	19	17	Na		<	1.5E+02
	Nvo 7.1E+01	A	4.9E-	+01	В	3.7E+01		С	4.1E	+01	N	1.8E	+08	R		>	1.2E+05
	Ver	ification of Methodology		Passed	Log10 I	Reductions/	cfu/ml										
N is	between 1.5E+8 cf	u/ml and 5E+8 cfu/ml, N =	1.8E+08	Yes													
Nv is b	etween 3E+2 cfu/m	nl and 1.6E+3 cfu/ml, Nv =	7.1E+02	Yes	Dirty		5.07										
	CLEAN A \geq 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	1												
	DIRTY C≥0.	5 x Nvo when 0.5 x Nvo =	1.9E+01	Yes	1												

				VALII	OITAC	NS										Test Proced	lura Ba	oulto
Test Organism	Bacterial	Experimental Condit	ions Valid	ation	Neu	utraliser T	oxicity	Di	ilution Neutra	isation Co	ntrol	Bacteria	al Test Sus	pension		Test Troceu		Suits
	Suspension	Clean	Di	rty	1	Contro	d		Clean	Dir	ty					Clean		Dirty
S.aureus	62 81	Vc	46	52	Vc	49	54	Vc		40	41	10-6	204	211	Vc		> 3	000 3000
0.5g/L												10-7	18	16	Na		<	3.0E+04
-	Nvo 7.2E+01	A	4.9E	+01	В	5.2E	+01	С		4.1E	+01	N	2.0E	+08	R		>	6.8E+02
	Ver	rification of Methodology		Passed	Log10	Reduction	ons/cfu/m	nl										
N is	between 1.5E+8 cf	u/ml and 5E+8 cfu/ml, N =	2.0E+08	Yes	Clean													
Nv is b	etween 3E+2 cfu/m	nl and 1.6E+3 cfu/ml, Nv =	7.2E+02	Yes	Dirty		2.83	3										
	CLEAN A \geq 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																	

1g/l Powder blend 1276.

Test							VALIDA	TIONS												Test Procedure Results				
Organis	Post	erial Suspension	E	xperimental Co	onditions	Validation (n	Moutral	ioor Toyl	city Contro	,	Dilution No	outralisatio	n Control		Bacteria	l Test Sus	pension			Test Proced	ure Results	*	
m	Bacil	eriai Suspension	Clean			Dirty		Neutrai	isei ioxi	City Contro	,	Cle	an	Dii	rty				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							B 6.1E+01			С	3.5E	+01	+01 5.3E+01		N	1.68	E+08	R	>	1.1E+05	>	1.1E+05	
	Verification of Methodology Passed									s/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, N	v = 7.8E +	02 Yes	Dirty 5.03																
			CLEAN A	≥ 0.5 x Nvo when	en 0.5 x N	vo = 3.9E+	-01 Yes																	
		DIRT	Y A ≥ 0.5	x Nvo when 0.5	.5 x Nvo=	3.9E+0	1 Yes																	
	$B \ge 0.5 \times Nvo \text{ when } 0.5 \times Nvo = 3.9E+01 \text{ Yes}$																							
		Yes																						
		DIRT	l Yes																					
																		•						

Test							VALID	ATIONS											1	Test Procedure Results						
Organis	D	terial Suspension		Experimental Conditions Validation					Neutraliser Toxicity Control Dilution N				utralisatio	n Control		Bacteria	pension	Test Proce				Julie Results				
m	Dati	teriai ousperision		Cle	Dirty		Neutra	iisei Toxicit	Control	Clean			Di	rty							Clean	Dirty				
E.hirae		67 60	Vo	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		С	4.2E+01		5.1E	+01	N	1.7E+08		R	>		1.1E+05	>	1.1E+0)5	
		,	erific	ation of Methodol	logy		Passed	Log10 F	teductions/c	fu/ml																
	N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N = 1.7E+08 Yes									5.04	4															
		Nv is between	en 3E	E+2 cfu/ml and 1.6E	+3 cfu/ml,	Nv = 6.4E	+02 Yes	Dirty		5.04	4															
			EAN A ≥ 0.5 x Nvo v	+01 Yes																						
	DIRTY A ≥ 0.5 x Nvo when 0.5 x Nvo= 3.2E+01 Yes																									
$B \ge 0.5 \times \text{Nvo when } 0.5 \times \text{Nvo} = 3.2E + 01$ Yes																										
CLEAN C \geq 0.5 Nvo when 0.5 x Nvo = 2.7E+01 Yes																										
		DI	$C \ge 0.5 \times Nvo when 0$	Yes																						

Test	Т		VALIDATIONS														1	Test Procedure Results				
Organis	: 🗆	Bacterial Suspension		Experimenta	s Validation		Moutral	oor Toylol	hi Cantral		Dilution Neutralisati	on Control	Bacteria	al Test Sus	pension			Test Froced	dure Results			
m		Bacterial Suspension		CI	ean	Dirty		Neutrai	eutraliser Toxicity Control			Clean	Dirty				Clean			Dirty		
P.aerugir	nosa	81 64	\	/c 42	36			Vc	42	45	Vc	34 31		10-6	216	212	Vc	<	15 15			
	1													10-7	21	22	Na	<	1.5E+02			
	Nv	/o 7.3E+01	Α	3.9	E+01			В	4.4	E+01	С	3.3E+01		N	2.1E	+08	R	>	1.4E+05			
			Verifi	cation of Methodo	logy		Passed	Log10 R	ductions/	cfu/ml												
		N is betwee	n 1.5E	+8 cfu/ml and 5E+8	cfu/ml, N =	2.1E+08	Yes	Clean		5.1	5			1								
		Nv is between	3E+2	cfu/ml and 1.6E+3	cfu/ml, Nv =	7.3E+02	Yes							1								
		CI	EAN	A ≥ 0.5 x Nvo when	0.5 x Nvo =	3.6E+01	Yes															
			DIRTY	$A \ge 0.5 \text{ x Nvo when}$	0.5 x Nvo=	3.6E+01	Yes															
			- 1	B ≥ 0.5 x Nvo when	0.5 x Nvo =	3.6E+01	Yes															
			CLEAN	I C ≥ 0.5 Nvo when	0.5 x Nvo =	2.2E+01	Yes							1								
			IRTY	$C \ge 0.5 \times Nvo when$	0.5 x Nvo=	2.2E+01	Yes															

Test								VALIDA	TIONS										Test Procedure Results					
Organis				Experimental Conditions Validation					Mandaell	T	- t Ot I		Dilution Ne	utralisatio	on Control	Bacteria	l Test Sus	pension				ure Results		
m	Bacterial Suspension		sion	Clean			Dirty		Neutrali	raliser Toxicity Control			Clean		Dirty				Clean			ean		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	<	1.5	E+02		
	Nvo	7.4E+	01	A	4.1E+0	1			В	4	4.4E+01	С	3.0E	+01		N	1.8E	+08	R	>	1.2	E+05		
			Ve	ification of	Methodology	1		Passed	Log10 Re	duction	s/cfu/ml													
		N is be	ween 1.	5E+8 cfu/ml	and 5E+8 cfu/	/ml, N =	1.8E+08	Yes	Clean		5.0	8												
		Nv is bet	veen 3E	+2 cfu/ml an	nd 1.6E+3 cfu/m	nl, Nv =	7.4E+02	Yes																
			CLEA	N A ≥ 0.5 x	Nvo when 0.5 >	x Nvo =	3.7E+01	Yes																
			DIR*	ΓY A ≥ 0.5 x	Nvo when 0.5	x Nvo=	3.7E+01	Yes																
				$B \ge 0.5 \text{ x}$	Nvo when 0.5 x	Nvo =	3.7E+01	Yes																
			CLE	AN C ≥ 0.5	Nvo when 0.5 x	x Nvo=	2.2E+01	Yes																
			DIRT	Y C ≥ 0.5 x	Nvo when 0.5	x Nvo=	2.2E+01	Yes																