Test Report for a General-Purpose Disinfectant Product
BS EN 1276:2019

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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. step1)

Company Name:	Sanzonate Europe Limited
Contact Name:	John Little
Contact Email:	john.little@sanzonate.com
Purchase Order No:	N/A
Report Date:	01/02/2024
Melbec Ref Number:	66448, 48437
Name of Test Product:	Sanzonate Ozone Solution
Batch Number:	Produced on Day of Test

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Sample Details:

Manufacture / Supplier:…...……………………………………………………	Sanzonate Europe Limited
Product storage conditions:…..………………………………………....	Ambient
Appearance of the product (as supplied):………………………………….	Clear colourless liquid
Appearance of the product (after dilution):…………………………………	N/A
Appearance of product with interfering substance and test organism:	Clear colourless liquid
Active substance and concentration:…...…………………………………	Ozone
Product dilution preparation…..……………………………………………	N/A
Product dilutions/concentrations:…..………………………………………	RTU (Ready to Use)
Diluent used to dilute product:…..…………………………………………	N/A
Incubation temperature: …..…………………………………………	35 ^{o}C to 38 ^{o}C

The test product for testing when received.
Date product received: 04/01/24   Test Date: 17/11/2024, 10/01/2024

Experimental Conditions:

Interfering substance:	N/A
Test temperature:	19 to 21 ^{o}C
Contact time:	5 minutes
Test organisms:	*Pseudomonas aeruginosa ATCC 15442*
	Staphylococcus aureus ATCC 6538
	Escherichia coli ATCC 10536
	Enterococcus hirae ATCC 10541

Deviations:

EN1276 states incubation temperature of 36±1°C or 37±1°C. Melbec Microbiology Ltd method states 35°C - 38°C.
The test product was tested at one concentration only at the clients request hence the testing is based on the test method of EN1276.
The client requested the test suspension inoculations of 1 log higher than the EN1276 standard stipulates and adapted clean conditions of
1ml inoculum in 99ml product.
The test organisms were prepared in phosphate buffered saline as per the clients request.

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Requirements of the Standard:

The test product shall demonstrate at least a 5 decimal logarithm (lg) reduction when tested in accordance with this standard under simulated clean or dirty conditions.

Conclusion:

For the product Sanzonate Ozone Solution, [Batch code: Produced on Day of Test] the log reduction requirements as specified in EN1276:2019 (5 lg within the relevant contact time) were met in adapted clean conditions with a contact time of 5 minutes. The client requested the test suspension inoculations of 1 log higher than the EN1276 standard stipulates and adapted clean conditions of 1ml inoculum in 99ml product.

Report authorised by:

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Name:	Nigel Mellors
Position:	Managing Director
Date:	01/02/2024

Name:	Victoria Wells
Position:	Biocides Lab Manager
Date:	01/02/2024

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Test Results:

Neutralisation Method Used:

Dilution neutralisation by pour plate

Neutraliser used   N1

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Pseudomonas aeruginosa ATCC 15442

	Validation and controls		Melbec Ref No	66448

Validation suspension (Nv_0)			Experimental conditions control (A)			Neutralizer control (B)			Method validation (C)		
Vc 1	93	$\bar{x} =$	Vc 1	86	$\bar{x} =$	Vc 1	72	$\bar{x} =$	Vc 1	68	$\bar{x} =$
Vc 2	84	88.5	Vc 2	82	84	Vc 2	68	70	Vc 2	74	71
$30 \le \bar{x}$ of $Nv_0 \le 160$?			$\bar{x}$ of A is $\ge 0.5 \times \bar{x}$ of Nv_0?			$\bar{x}$ of B is $\ge 0.5 \times \bar{x}$ of Nv_0?			$\bar{x}$ of C is $\ge 0.5 \times \bar{x}$ of Nv_0?		
Yes			**Yes**			**Yes**			**Yes**		

Test suspension

Test suspension (N and N_0):	N	Vc 1	Vc 2			
	10^{-7}	>330	>330	X m	3.80E+09	; lg N = 9.58
				$N_0 = N/10$; lg N_0=	8.58	
	10^{-8}	43	33	$8.17 \le$ lg$N_0 \le 8.70$?	Yes	
				$\bar{x}$ quotient = >5 and <15?	N/A	

Bactericidal activity results

PPM of Product	Conc. of the active (%)	Vc 1	Vc 2	$Na = \bar{x} \times 10$	lgNa	lg$R$ $N_0 =$ 8.58	Contact time	Result
1.8	1ml of inoculum into 99ml product	<14	<14	1.40E+02	<2.15	>6.43	5 minutes	**Pass**

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Staphylococcus aureus ATCC 6538

| | **Validation and controls** | | **Melbec Ref No** | **66448** |

Validation and controls

Validation suspension (Nv_0)			Experimental conditions control (**A**)			Neutralizer control (**B**)			Method validation (**C**)		
Vc 1	102	$\overline{x}$ =	Vc 1	94	$\overline{x}$ =	Vc 1	88	$\overline{x}$ =	Vc 1	84	$\overline{x}$ =
Vc 2	99	100.5	Vc 2	90	92	Vc 2	85	86.5	Vc 2	72	78
$30 \leq \overline{x}$ of $Nv_0 \leq 160$?			$\overline{x}$ of A is ≥ 0.5 x $\overline{x}$ of Nv_0?			$\overline{x}$ of B is ≥ 0.5 x $\overline{x}$ of Nv_0?			$\overline{x}$ of C is ≥ 0.5 x $\overline{x}$ of Nv_0?		
Yes			**Yes**			**Yes**			**Yes**		

Test suspension

		N	**Vc 1**	**Vc 2**			
Test suspension (**N** and **N**$_0$):					X m	1.51E+09	; lg N = 9.18
		10^{-7}	>330	>330	$N_0 = N/10$; lg N_0=		8.18
		10^{-8}	35	30	$8.17 \leq \lg N_0 \leq 8.70$?		Yes
					$\overline{x}$ quotient = >5 and <15?		N/A

Bactericidal activity results

PPM of Product	Conc. of the active (%)	Vc 1	Vc 2	$Na = \overline{x} \, x10$	lgNa	lg$R$ $N_0 =$ 8.18	Contact time	Result
1.75	1ml of inoculum into 99ml product	<14	<14	1.40E+02	<2.15	>6.03	5 minutes	**Pass**

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Escherichia coli ATCC 10536

				Melbec Ref No	66448
		Validation and controls			

Validation suspension (Nv_0)			Experimental conditions control (**A**)			Neutralizer control (**B**)			Method validation (**C**)		
Vc 1	74	$\overline{X}$ =	Vc 1	72	$\overline{X}$ =	Vc 1	66	$\overline{X}$ =	Vc 1	36	$\overline{X}$ =
Vc 2	56	65	Vc 2	62	67	Vc 2	31	48.5	Vc 2	38	37
$30 \le \overline{X}$ of $Nv_0 \le 160$?			$\overline{X}$ of A is ≥ 0.5 x $\overline{X}$ of Nv_0?			$\overline{X}$ of B is ≥ 0.5 x $\overline{X}$ of Nv_0?			$\overline{X}$ of C is ≥ 0.5 x $\overline{X}$ of Nv_0?		
Yes			**Yes**			**Yes**			**Yes**		

Test suspension

Test suspension (**N** and N_0):		**N**	Vc 1	Vc 2	X wm	2.20E+09	; lg N =	9.34
		10^{-7}	225	212	$N_0 = N/10$; lg N_0=			8.34
		10^{-8}	28	20	$8.17 \le lgN_0 \le 8.70$?		Yes	
					$\overline{X}$ quotient = >5 and <15?			9.10

Bactericidal activity results

PPM of Product	Conc. of the active (%)	Vc 1	Vc 2	$Na = \overline{X}$ x10	lgNa	lg**R** N_0 =		Contact time	Result
2.15	1ml of inoculum into 99ml product	<14	<14	1.40E+02	<2.15		>6.20	5 minutes	**Pass**

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Enterococcus hirae ATCC 10541

	Validation and controls		Melbec Ref No	66448

Validation and controls

Validation suspension (Nv_0)			Experimental conditions control (A)			Neutralizer control (B)			Method validation (C)		
Vc 1	133	$\bar{x}$ =	Vc 1	106	$\bar{x}$ =	Vc 1	122	$\bar{x}$ =	Vc 1	90	$\bar{x}$ =
Vc 2	120	126.5	Vc 2	113	109.5	Vc 2	100	111	Vc 2	65	77.5
$30 \leq \bar{x}$ of $Nv_0 \leq 160$?			$\bar{x}$ of A is ≥ 0.5 x $\bar{x}$ of Nv_0?			$\bar{x}$ of B is ≥ 0.5 x $\bar{x}$ of Nv_0?			$\bar{x}$ of C is ≥ 0.5 x $\bar{x}$ of Nv_0?		
Yes			**Yes**			**Yes**			**Yes**		

Test suspension

		N	Vc 1	Vc 2			
Test suspension (N and N_0):		10^{-7}	>330	>330	X m	4.95E+09 ; lg N =	9.69
					$N_0 = N/10$; lg N_0=		8.69
		10^{-8}	51	48	$8.17 \leq$ lg$N_0 \leq 8.70$?		Yes
					$\bar{x}$ quotient = >5 and <15?		N/A

Bactericidal activity results

PPM of Product	Conc. of the active (%)	Vc 1	Vc 2	$Na = \bar{x} \, x10$	lgNa	lg$R$ N_0 =	8.69	Contact time	Result
1.71	1ml of inoculum into 99ml product	59	49	5.40E+02	2.73		5.96	5 minutes	**Pass**

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