Report: SSL.19B055.MB3 **Issued:** 5 April 2019 **Page:** 1 of 7

Test Report: EN 13727:2012+A2:2015

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity in the medical area – Test method and requirements (phase 2, step 1)

Identification of the test laboratory:	Abbott Analytical Ltd Unit 2, Hickmans Road, Birkenhead, CH41 1JH, United Kingdom
Identification of the client:	Safe Solutions (Safe4) Ltd Wharton Green, Bostock Road, Winsford, CW7 3BD, United Kingdom
Identification of the sample:	19B/055
Name of the product:	Safe4 Instrument Disinfectant
Batch number/reference and expiry date (if available):	3060
Date of delivery:	25 February 2019
Storage conditions:	Room temperature in darkness
Product diluent recommended by the manufacturer for use:	Not disclosed
Active substance(s) and their concentrations (s) (optional):	Not disclosed
Appearance of the product:	Clear green liquid

Notes:

1) The test results in this report relate only to the sample(s) tested.
2) This test report may not be reproduced except in full, adapted, altered or used to create a derivative work, without written approval from Abbott Analytical Ltd.

Test method and its validation:

Method:	Dilution-neutralisation
Neutraliser:	30.0 g/l Polysorbate 80 + 3.0 g/l Lecithin + 1.0 g/l L-histidine + 1.0 g/l L-cysteine (Neutraliser A) 100.0 g/l Polysorbate 80 + 30.0 g/l Lecithin + 30.0 g/l Tryptone Soya Broth + 5.0 g/l Sodium thiosulphate + 1.0 g/l L-histidine (Neutraliser B)
Neutraliser validation:	Validated in accordance with EN 13727:2012+A2:2015 (5.5.2)

Experimental conditions:

Period of analysis:	12 March 2019 to 4 April 2019
Product test concentration(s):	1:20
Diluent used for product test solution(s):	Hard water
Contact time(s):	10 min ± 10 s
Test temperature(s):	20°C ± 1°C
Interfering substance:	0.3 g/l bovine albumin (clean conditions)
Temperature of incubation:	36°C ± 1°C
Identification of the bacterial strain(s) used:	*Pseudomonas aeruginosa* (NCIMB 10421) *Staphylococcus aureus* (NCTC 10788) *Enterococcus hirae* (NCIMB 8192)

Deviations: None

Remarks:

1) All test conditions are as requested by the client, irrespective of whether these are in accordance with EN 13727:2012+A2:2015 (5.4.2) or EN 13727:2012+A2:2015 (5.5.1.1).

Requirements:

The product shall demonstrate at least a 5 decimal log (lg) reduction against every test organism.

Conclusion:

According to EN 13727:2012+A2:2015, Safe4 Instrument Disinfectant possesses bactericidal activity when tested at a concentration of 1:20 with a contact time of 10 minutes at 20°C under clean conditions against all of the referenced strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus hirae*.

Report prepared by:

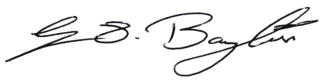
Signed: 

Name: Tony Watson

Position: General Manager

Date: 5 April 2019

Approved by:

Signed: 

Name: Gareth Bayliss

Position: Laboratory Manager

Date: 5 April 2019

Results: EN 13727:2012+A2:2015

Test organism:	*Pseudomonas aeruginosa*		(NCIMB 10421)
Date of test:	12 March 2019		
Test temperature:	20°C ± 1°C	Incubation temperature:	36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	A	Test conditions:	Clean conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *1:20*		
*Vc*1	*50*	$\bar{\kappa}$ =	*Vc*1	*41*	$\bar{\kappa}$ =	*Vc*1	*52*	$\bar{\kappa}$ =	*Vc*1	*53*	$\bar{\kappa}$ =
*Vc*2	*54*	52	*Vc*2	*49*	45	*Vc*2	*54*	53	*Vc*2	*50*	51.5
30 ≤ $\bar{\kappa}$ of Nv_o ≤ 160 ? ☒ yes ☐ no			$\bar{\kappa}$ of *A* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ? ☒ yes ☐ no			$\bar{\kappa}$ of *B* ≥ 0.5 x $\bar{\kappa}$ of Nv_o (or Nv_B / 1000) ? ☒ yes ☐ no			$\bar{\kappa}$ of *C* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ? ☒ yes ☐ no		
Validation suspension (Nv_B)			*Vc*1	*52*	$\bar{\kappa}$ =	30 ≤ $\bar{\kappa}$ of Nv_B / 1000 ≤ 160 ? ☒ yes ☐ no					
			*Vc*2	*51*	51.5						

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2		
10^{-6}	*>330*	*>330*	$\bar{\kappa}$ wm = 4.10×10^{8} ;	lg *N* = 8.61
10^{-7}	*39*	*43*	$N_o = N / 10$;	lg N_o = 7.61
			7.17 ≤ lg N_o ≤ 7.70 ? ☒ yes	☐ no

Test:

Conc. of the product	Contact time	Dilution step	*Vc*1	*Vc*2	*Na* ($\bar{\kappa}$ x 10 or $\bar{\kappa}$ wm x 10)	lg *Na*	lg *R* (lg N_o - lg *Na*)
1:20	*10 min*	10^{0}	*0*	*0*	<140	<2.15	>5.46
		10^{-1}	*0*	*0*			

Results: EN 13727:2012+A2:2015

Test organism:	*Staphylococcus aureus*		(NCTC 10788)
Date of test:	2 April 2019		
Test temperature:	20°C ± 1°C	Incubation temperature:	36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	B	Test conditions:	Clean conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *1:20*		
*Vc*1	*70*	$\bar{n}$ =	*Vc*1	*70*	$\bar{n}$ =	*Vc*1	*65*	$\bar{n}$ =	*Vc*1	*64*	$\bar{n}$ =
*Vc*2	*72*	71	*Vc*2	*68*	69	*Vc*2	*72*	68.5	*Vc*2	*73*	68.5
30 ≤ $\bar{n}$ of Nv_o ≤ 160 ? ☒ yes ☐ no			$\bar{n}$ of *A* ≥ 0.5 x $\bar{n}$ of Nv_o ? ☒ yes ☐ no			$\bar{n}$ of *B* ≥ 0.5 x $\bar{n}$ of Nv_o (or Nv_B / 1000) ? ☒ yes ☐ no			$\bar{n}$ of *C* ≥ 0.5 x $\bar{n}$ of Nv_o ? ☒ yes ☐ no		
Validation suspension (Nv_B)			*Vc*1	*69*	$\bar{n}$ =	30 ≤ $\bar{n}$ of Nv_B / 1000 ≤ 160 ? ☒ yes ☐ no					
			*Vc*2	*72*	70.5						

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2		
10^{-6}	*324*	*319*	$\bar{n}$ wm = 3.23×10^{8} ;	lg *N* = 8.51
10^{-7}	*32*	*35*	$N_o = N / 10$;	lg N_o = 7.51
			7.17 ≤ lg N_o ≤ 7.70 ? ☒ yes	☐ no

Test:

Conc. of the product	Contact time	Dilution step	*Vc*1	*Vc*2	*Na* ($\bar{n}$ x 10 or $\bar{n}$ wm x 10)	lg *Na*	lg *R* (lg N_o - lg *Na*)
1:20	*10 min*	10^{0}	*0*	*0*	<140	<2.15	>5.36
		10^{-1}	*0*	*0*			

Results: EN 13727:2012+A2:2015

Test organism:	*Enterococcus hirae*		(NCIMB 8192)
Date of test:	2 April 2019		
Test temperature:	20°C ± 1°C	Incubation temperature:	36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	B	Test conditions:	Clean conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *1:20*		
*Vc*1	*40*	$\bar{\kappa}$ =	*Vc*1	*39*	$\bar{\kappa}$ =	*Vc*1	*45*	$\bar{\kappa}$ =	*Vc*1	*44*	$\bar{\kappa}$ =
*Vc*2	*43*	41.5	*Vc*2	*39*	39	*Vc*2	*37*	41	*Vc*2	*40*	42
$30 \le \bar{\kappa}$ of $Nv_o \le 160$? ☒ yes ☐ no			$\bar{\kappa}$ of $A \ge 0.5 \times \bar{\kappa}$ of Nv_o ? ☒ yes ☐ no			$\bar{\kappa}$ of $B \ge 0.5 \times \bar{\kappa}$ of Nv_o (or $Nv_B / 1000$) ? ☒ yes ☐ no			$\bar{\kappa}$ of $C \ge 0.5 \times \bar{\kappa}$ of Nv_o ? ☒ yes ☐ no		
Validation suspension (Nv_B)			*Vc*1	*39*	$\bar{\kappa}$ =	$30 \le \bar{\kappa}$ of $Nv_B / 1000 \le 160$? ☒ yes ☐ no					
			*Vc*2	*40*	39.5						

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2	$\bar{\kappa}$ wm = 1.99×10^8 ; lg *N* = 8.30
10^{-6}	*198*	*199*	$N_o = N / 10$; lg N_o = 7.30
10^{-7}	*21*	*19*	$7.17 \le \lg N_o \le 7.70$? ☒ yes ☐ no

Test:

Conc. of the product	Contact time	Dilution step	*Vc*1	*Vc*2	*Na* ($\bar{\kappa}$ x 10 or $\bar{\kappa}$ wm x 10)	lg *Na*	lg *R* (lg N_o - lg *Na*)
1:20	*10 min*	10^0	*0*	*0*	<140	<2.15	>5.15
		10^{-1}	*0*	*0*			

Explanations:

Vc	count per ml (one plate or more)
$\overline{n}$	average of *Vc*1 and *Vc*2 (1 + 2 duplicate)
$\overline{n}$ wm	weighted mean of $\overline{n}$
N	number of cells per ml in the test suspension
N_o	number of cells in the test mixture at the beginning of the contact time ($N_o = N / 10$)
Na	number of survivors per ml in the test mixture at the end of the contact time (before neutralisation or filtration)
R	reduction ($\lg R = \lg N_o - \lg Na$)
Nv	number of cells per ml in the validation suspension
Nv_o	number of cells in the validation mixtures at the beginning of the contact time ($Nv_o = Nv / 10$)
A	number of survivors per ml in the experimental conditions control mixture at the end of the contact time
B	number of survivors per ml in the neutraliser or filtration control mixture after 5 minutes
C	number of survivors per ml in the method validation mixture after 30 minutes