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/023
Name of the product:	Handwash
Batch number/reference and expiry date (if available):	3442
Date of delivery:	18 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:	Thick clear colourless 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:	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:	27 February 2019 to 1 March 2019
Product test concentration(s):	50%
Diluent used for product test solution(s):	Hard water
Contact time(s):	30 s ± 5 s
Test temperature(s):	20°C ± 1°C
Interfering substance:	3.0 g/l bovine albumin + 3.0 ml/l sheep erythrocytes (dirty conditions)
Temperature of incubation:	36°C ± 1°C
Identification of the bacterial strain(s) used:	*Pseudomonas aeruginosa* (DSM 939) *Escherichia coli* K12 (NCIMB 10083) *Staphylococcus aureus* (DSM 799) *Enterococcus hirae* (DSM 3320)

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).
2) Handwash products are always pre-diluted with hard water. This solution simulates the addition of tap water in practice (1:1).

Requirements:

The product shall demonstrate at least a 3 decimal log (lg) reduction against all test organisms.

Conclusion:

According to EN 13727:2012+A2:2015,this sample of Handwash possesses bactericidal activity when tested at a concentration of 50% with a contact time of 30 seconds at 20°C under dirty conditions against all of the referenced strains of *Pseudomonas aeruginosa*, *Escherichia coli* K12, *Staphylococcus aureus* and *Enterococcus hirae*.

Report prepared by:

Signed: 

Name: Tony Watson

Position: General Manager

Date: 5 March 2019

Approved by:

Signed: 

Name: Gareth Bayliss

Position: Laboratory Manager

Date: 5 March 2019

Abbott Analytical Ltd
Unit 2, Hickmans Road,
Birkenhead, CH41 1JH, United Kingdom

T: +44 (0)151 345 6753
E: enqs@abbottanalytical.co.uk
W: www.abbottanalytical.co.uk

Results: EN 13727:2012+A2:2015

Test organism:	*Pseudomonas aeruginosa*		(DSM 939)
Date of test:	27 February 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:	Dirty conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *50%*		
*Vc*1	*74*	$\bar{n}$ =	*Vc*1	*71*	$\bar{n}$ =	*Vc*1	*75*	$\bar{n}$ =	*Vc*1	*74*	$\bar{n}$ =
*Vc*2	*79*	76.5	*Vc*2	*73*	72	*Vc*2	*79*	77	*Vc*2	*74*	74
$30 \le \bar{n}$ of $Nv_o \le 160$? ☒ yes ☐ no			$\bar{n}$ of *A* $\ge 0.5 \times \bar{n}$ of Nv_o ? ☒ yes ☐ no			$\bar{n}$ of *B* $\ge 0.5 \times \bar{n}$ of Nv_o (or $Nv_B / 1000$) ? ☒ yes ☐ no			$\bar{n}$ of *C* $\ge 0.5 \times \bar{n}$ of Nv_o ? ☒ yes ☐ no		
Validation suspension (Nv_B)			*Vc*1	*76*	$\bar{n}$ =	$30 \le \bar{n}$ of $Nv_B / 1000 \le 160$? ☒ yes ☐ no					
			*Vc*2	*79*	77.5						

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2		
10^{-6}	*>330*	*>330*	$\bar{n}$ wm = 4.40×10^8 ;	lg *N* = 8.64
10^{-7}	*42*	*46*	$N_o = N / 10$;	lg N_o = 7.64
			$7.17 \le \lg N_o \le 7.70$? ☒ yes ☐ no	

Test:

Conc. of the product	Contact time	Dilution step	*Vc*1	*Vc*2	*Na* ($\bar{n}$ x 10 or $\bar{n}$ wm x 10)	lg *Na*	lg *R* (lg N_o - lg *Na*)
50%	*30 s*	10^0	*4*	*0*	$<1.40 \times 10^2$	<2.15	>5.49
		10^{-1}	*0*	*0*			
		10^{-2}	*0*	*0*			

Results: EN 13727:2012+A2:2015

Test organism:	*Escherichia coli* K12		(NCIMB 10083)
Date of test:	27 February 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:	Dirty conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *50%*		
*Vc*1	*70*	$\bar{\kappa}$ =	*Vc*1	*71*	$\bar{\kappa}$ =	*Vc*1	*64*	$\bar{\kappa}$ =	*Vc*1	*69*	$\bar{\kappa}$ =
*Vc*2	*68*	69	*Vc*2	*67*	69	*Vc*2	*66*	65	*Vc*2	*69*	69
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	*70*	$\bar{\kappa}$ =	30 ≤ $\bar{\kappa}$ of Nv_B / 1000 ≤ 160 ? ☒ yes ☐ no							
	*Vc*2	*70*	70								

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2			
10^{-6}	*>330*	*>330*	$\bar{\kappa}$ wm = 4.25×10^{8} ;	lg *N* =	8.63
10^{-7}	*41*	*44*	N_o = *N* / 10 ;	lg N_o =	7.63
			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*)
50%	*30 s*	10^{0}	*0*	*0*	$<1.40 \times 10^{2}$	<2.15	>5.48
		10^{-1}	*0*	*0*			
		10^{-2}	*0*	*0*			

Results: EN 13727:2012+A2:2015

Test organism:	*Staphylococcus aureus*		(DSM 799)
Date of test:	27 February 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:	Dirty conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *50%*		
*Vc*1	*46*	$\bar{n}$ =	*Vc*1	*53*	$\bar{n}$ =	*Vc*1	*49*	$\bar{n}$ =	*Vc*1	*49*	$\bar{n}$ =
*Vc*2	*49*	47.5	*Vc*2	*50*	51.5	*Vc*2	*47*	48	*Vc*2	*53*	51
$30 \le \bar{n}$ of $Nv_o \le 160$? ☒ yes ☐ no			$\bar{n}$ of *A* $\ge 0.5 \times \bar{n}$ of Nv_o ? ☒ yes ☐ no			$\bar{n}$ of *B* $\ge 0.5 \times \bar{n}$ of Nv_o (or $Nv_B / 1000$) ? ☒ yes ☐ no			$\bar{n}$ of *C* $\ge 0.5 \times \bar{n}$ of Nv_o ? ☒ yes ☐ no		
Validation suspension (Nv_B)			*Vc*1	*48*	$\bar{n}$ =	$30 \le \bar{n}$ of $Nv_B / 1000 \le 160$? ☒ yes ☐ no					
			*Vc*2	*47*	47.5						

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2		
10^{-6}	*231*	*221*	$\bar{n}$ wm = 2.25×10^8 ;	lg *N* = 8.35
10^{-7}	*23*	*21*	$N_o = N / 10$;	lg N_o = 7.35
			$7.17 \le \lg N_o \le 7.70$? ☒ yes	☐ no

Test:

Conc. of the product	Contact time	Dilution step	*Vc*1	*Vc*2	*Na* ($\bar{n}$ x 10 or $\bar{n}$ wm x 10)	lg *Na*	lg *R* (lg N_o - lg *Na*)
50%	*30 s*	10^0	*127*	*119*	1.23×10^3	3.09	4.26
		10^{-1}	*12*	*13*			
		10^{-2}	*1*	*2*			

Results: EN 13727:2012+A2:2015

Test organism:	*Enterococcus hirae*		(DSM 3320)
Date of test:	27 February 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:	Dirty conditions

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *50%*		
*Vc*1	*40*	$\bar{n}$ =	*Vc*1	*41*	$\bar{n}$ =	*Vc*1	*37*	$\bar{n}$ =	*Vc*1	*49*	$\bar{n}$ =
*Vc*2	*43*	41.5	*Vc*2	*40*	40.5	*Vc*2	*42*	39.5	*Vc*2	*37*	43
$30 \le \bar{n}$ of $Nv_o \le 160$? ☒ yes ☐ no			$\bar{n}$ of $A \ge 0.5 \times \bar{n}$ of Nv_o ? ☒ yes ☐ no			$\bar{n}$ of $B \ge 0.5 \times \bar{n}$ of Nv_o (or $Nv_B / 1000$) ? ☒ yes ☐ no			$\bar{n}$ of $C \ge 0.5 \times \bar{n}$ of Nv_o ? ☒ yes ☐ no		
Validation suspension (Nv_B)	*Vc*1	*39*	$\bar{n}$ =	$30 \le \bar{n}$ of $Nv_B / 1000 \le 160$? ☒ yes ☐ no							
	*Vc*2	*37*	38								

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2	$\bar{n}$ wm = 3.01×10^8 ; lg *N* = 8.48
10^{-6}	*297*	*304*	$N_o = N / 10$; lg N_o = 7.48
10^{-7}	*29*	*33*	$7.17 \le \lg N_o \le 7.70$? ☒ yes ☐ no

Test:

Conc. of the product	Contact time	Dilution step	*Vc*1	*Vc*2	*Na* ($\bar{n}$ x 10 or $\bar{n}$ wm x 10)	lg *Na*	lg *R* (lg N_o - lg *Na*)
50%	*30 s*	10^0	*10*	*14*	$<1.40 \times 10^2$	<2.15	>5.33
		10^{-1}	*1*	*2*			
		10^{-2}	*0*	*0*			

Explanations:

Vc	count per ml (one plate or more)
$\overline{Vc}$	average of *Vc*1 and *Vc*2 (1 + 2 duplicate)
$\overline{Vc}$ wm	weighted mean of $\overline{Vc}$
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

Abbott Analytical Ltd
Unit 2, Hickmans Road,
Birkenhead, CH41 1JH, United Kingdom

T: +44 (0)151 345 6753
E: enqs@abbottanalytical.co.uk
W: www.abbottanalytical.co.uk