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Test Report: EN 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)

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: 19F/006

Name of the product:	PDSA Shampoo
Batch number/reference and expiry date (if available):	3671
Date of delivery:	7 June 2016
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 pink 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) or 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 1276:2009 (5.5.2)

Experimental conditions:

Period of analysis:	14 June 2019 to 18 July 2019
Product test concentration(s):	Neat
Diluent used for product test solution(s):	N/A
Contact time(s):	5 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) *Escherichia coli* (NCTC 10418) *Staphylococcus aureus* (NCTC 10788) *Enterococcus hirae* (NCIMB 8192)

Deviations:

1) Additional serial dilutions of *Na* – down to 10^{-2} – undertaken at client's request in order to show a greater than 3 lg reduction against each test organism.

Remarks:

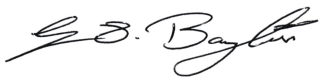
1) All test conditions are as requested by the client, irrespective of whether these are in accordance with EN 1276:2009 (5.4.2) or EN 1276:2009 (5.5.1.1).
2) Products can only be tested at a concentration of 80% or less as some dilution is always produced by adding the test organisms and interfering substance.

Requirements:

The client has requested that the product demonstrates at least a 3 decimal log (lg) reduction against every test organism.

Conclusion:

PDSA Shampoo achieves a greater than 3 lg reduction when tested neat with a contact time of 5 minutes at 20°C under clean conditions against all of the referenced strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus hirae*.

Report prepared by:		**Approved by:**	
Signed:		Signed:	
Name:	Tony Watson	Name:	Gareth Bayliss
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Date:	19 July 2019	Date:	19 July 2019

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Results: EN 1276:2009

Test organism:	*Pseudomonas aeruginosa*		(NCIMB 10421)
Date of test:	14 June 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.: *Neat*		
*Vc*1	*56*	$\bar{\kappa}$ =	*Vc*1	*61*	$\bar{\kappa}$ =	*Vc*1	*59*	$\bar{\kappa}$ =	*Vc*1	*55*	$\bar{\kappa}$ =
*Vc*2	*63*	59.5	*Vc*2	*60*	60.5	*Vc*2	*67*	63	*Vc*2	*57*	56
30 ≤ $\bar{\kappa}$ of Nv_o ≤ 160 ?			$\bar{\kappa}$ of *A* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ?			$\bar{\kappa}$ of *B* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ?			$\bar{\kappa}$ of *C* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2	$\bar{\kappa}$ wm = 4.45 x 10^8 ; lg *N* = 8.65
10^{-6}	*>330*	*>330*	N_o = *N* / 10 ; lg N_o = 7.65
10^{-7}	*46*	*43*	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*)
Neat	*5 min*	10^0	*>330*	*>330*	3.95 x 10^4	4.60	3.05
		10^{-1}	*>330*	*>330*			
		10^{-2}	*39*	*40*			

Results: EN 1276:2009

Test organism:	*Escherichia coli*		(NCTC 10418)
Date of test:	2 July 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.: *Neat*		
*Vc*1	*94*	$\bar{\kappa}$ =	*Vc*1	*91*	$\bar{\kappa}$ =	*Vc*1	*94*	$\bar{\kappa}$ =	*Vc*1	*91*	$\bar{\kappa}$ =
*Vc*2	*89*	91.5	*Vc*2	*99*	95	*Vc*2	*93*	93.5	*Vc*2	*92*	91.5
30 ≤ $\bar{\kappa}$ of Nv_o ≤ 160 ?			$\bar{\kappa}$ of *A* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ?			$\bar{\kappa}$ of *B* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ?			$\bar{\kappa}$ of *C* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2	$\bar{\kappa}$ wm = 3.60 x 10^8 ; lg *N* = 8.56
10^{-6}	*>330*	*>330*	N_o = *N* / 10 ; lg N_o = 7.56
10^{-7}	*34*	*38*	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*)
Neat	*5 min*	10^0	*>330*	*>330*	2.46 x 10^4	4.39	3.17
		10^{-1}	*254*	*243*			
		10^{-2}	*24*	*20*			

Results: EN 1276:2009

Test organism:	*Staphylococcus aureus*		(NCTC 10788)
Date of test:	16 July 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.: *Neat*		
*Vc*1	*59*	$\bar{\kappa}$ =	*Vc*1	*61*	$\bar{\kappa}$ =	*Vc*1	*60*	$\bar{\kappa}$ =	*Vc*1	*56*	$\bar{\kappa}$ =
*Vc*2	*63*	61	*Vc*2	*65*	63	*Vc*2	*54*	57	*Vc*2	*55*	55.5
30 ≤ $\bar{\kappa}$ of Nv_o ≤ 160 ?			$\bar{\kappa}$ of *A* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ?			$\bar{\kappa}$ of *B* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ?			$\bar{\kappa}$ of *C* ≥ 0.5 x $\bar{\kappa}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2	$\bar{\kappa}$ wm = 4.50 x 10^8 ; lg *N* = 8.65
10^{-6}	*>330*	*>330*	N_o = *N* / 10 ; lg N_o = 7.65
10^{-7}	*44*	*46*	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*)
Neat	*5 min*	10^0	*>330*	*>330*	4.00 x 10^3	3.60	4.05
		10^{-1}	*41*	*39*			
		10^{-2}	*4*	*5*			

Results: EN 1276:2009

Test organism:	*Enterococcus hirae*		(NCIMB 8192)
Date of test:	2 July 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.: *Neat*		
*Vc*1	*117*	$\overline{\kappa}$ =	*Vc*1	*119*	$\overline{\kappa}$ =	*Vc*1	*124*	$\overline{\kappa}$ =	*Vc*1	*123*	$\overline{\kappa}$ =
*Vc*2	*118*	117.5	*Vc*2	*127*	123	*Vc*2	*122*	123	*Vc*2	*130*	126.5
30 ≤ $\overline{\kappa}$ of Nv_o ≤ 160 ?			$\overline{\kappa}$ of *A* ≥ 0.5 x $\overline{\kappa}$ of Nv_o ?			$\overline{\kappa}$ of *B* ≥ 0.5 x $\overline{\kappa}$ of Nv_o ?			$\overline{\kappa}$ of *C* ≥ 0.5 x $\overline{\kappa}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2	$\overline{\kappa}$ wm = 4.60×10^8 ; lg *N* = 8.66
10^{-6}	*>330*	*>330*	$N_o = N / 10$; lg N_o = 7.66
10^{-7}	*47*	*45*	7.17 ≤ lg N_o ≤ 7.70 ? ☒ yes ☐ no

Test:

Conc. of the product	Contact time	Dilution step	*Vc*1	*Vc*2	*Na* ($\overline{\kappa}$ x 10 or $\overline{\kappa}$ wm x 10)	lg *Na*	lg *R* (lg N_o - lg *Na*)
Neat	*5 min*	10^0	*>330*	*>330*	3.85×10^3	3.59	4.07
		10^{-1}	*40*	*37*			
		10^{-2}	*4*	*3*			

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Explanations:

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

All test results have an associated uncertainty of measurement, details of which are available on request.

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