Test Report: EN 1656:2019

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

Identification of the test laboratory: Abbott Analytical Ltd
Unit 2, Hickmans Road, Birkenhead, CH41 1JH, Great Britain

Identification of the client: Safe Solutions (Safe4) Ltd
Wharton Green, Bostock Road, Winsford, CW7 3BD, Great Britain

Identification of the sample: 24K/055

Name of the product:	Safe4 Disinfectant Concentrate
Batch number/reference and expiry date (if available):	0053
Date of delivery:	30 October 2024
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 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.
3) Re-issue of report SSL.24K055.VB, dated 15 November 2024.
Reason: Change of product name.

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 1656:2019 (5.5.2)

Experimental conditions:

Period of analysis:	12 November 2024 to 14 November 2024
Product test concentration(s):	1:50 (1 part product to 50 parts water) v/v
Diluent used for product test solution(s):	Hard water
Contact time(s):	5 min ± 10 s
Test temperature(s):	10°C ± 1°C
Interfering substance:	10.0 g/l bovine albumin + 10.0 g/l yeast extract (high-level soiling)
Temperature of incubation:	36°C ± 1°C
Identification of the bacterial strain(s) used:	*Proteus hauseri* (NCTC 4175) *Staphylococcus aureus* (NCTC 10788) *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 1656:2019 (5.4.2) or EN 1656:2019 (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 1656:2019, this sample of Safe4 Disinfectant Concentrate possesses bactericidal activity against the referenced strains of *Proteus hauseri*, *Staphylococcus aureus* and *Enterococcus hirae*, when tested at a concentration of 1:50 (1 part product to 50 parts water) with a contact time of 5 minutes at 10°C under high-level soiling.

Approved by:

Signed: 

Name: Tony Watson

Position: General Manager

Date: 12 December 2024

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

Registered address: 128 City Road,
London, EC1V 2NX, United Kingdom

A company registered in England and Wales
Company number: 10031406

Results: EN 1656:2019

RST 003 (Issue 6)

Test organism:	*Proteus hauseri*		(NCTC 4175)
Date of test:	12 November 2024	Test temperature:	10°C ± 1°C
Interfering substance:	10.0 g/l bovine albumin + 10.0 g/l yeast extract		
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	B	Incubation temperature:	36°C ± 1°C

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (A)			Neutraliser or filtration control (B)			Method validation (C) Product conc.: *1:50*		
*Vc*1	*75*	$\overline{n}$ =	*Vc*1	*60*	$\overline{n}$ =	*Vc*1	*73*	$\overline{n}$ =	*Vc*1	*65*	$\overline{n}$ =
*Vc*2	*97*	86	*Vc*2	*71*	65.5	*Vc*2	*67*	70	*Vc*2	*86*	75.5
$30 \le \overline{n}$ of $Nv_o \le 160$?			$\overline{n}$ of $A \ge 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $B \ge 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $C \ge 0.5 \times \overline{n}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (N and N_o):

N	*Vc*1	*Vc*2	$\overline{n}_{wm}$ = 4.70×10^8 ; lg N = 8.67
10^{-6}	*>330*	*>330*	$N_o = N / 10$; lg N_o = 7.67
10^{-7}	*48*	*46*	$7.17 \le \lg N_o \le 7.70$? ☒ yes ☐ no

Test:

Conc. of the product	Contact time	*Vc*1	*Vc*2	*Na* ($\overline{n} \times 10$)	lg *Na*	lg *R* (lg N_o - lg *Na*)
1:50	*5 min*	*0*	*0*	<140	<2.15	>5.52

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

Registered address: 128 City Road,
London, EC1V 2NX, United Kingdom

A company registered in England and Wales
Company number: 10031406

Results: EN 1656:2019

RST 003 (Issue 6)

Test organism:	*Staphylococcus aureus*		(NCTC 10788)
Date of test:	12 November 2024	Test temperature:	10°C ± 1°C
Interfering substance:	10.0 g/l bovine albumin + 10.0 g/l yeast extract		
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	B	Incubation temperature:	36°C ± 1°C

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *1:50*		
*Vc*1	*57*	$\overline{n}$ =	*Vc*1	*58*	$\overline{n}$ =	*Vc*1	*62*	$\overline{n}$ =	*Vc*1	*53*	$\overline{n}$ =
*Vc*2	*54*	55.5	*Vc*2	*64*	61	*Vc*2	*55*	58.5	*Vc*2	*44*	48.5
$30 \le \overline{n}$ of $Nv_o \le 160$?			$\overline{n}$ of $A \ge 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $B \ge 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $C \ge 0.5 \times \overline{n}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2			
10^{-6}	*190*	*180*	$\overline{n}_{wm}$ = 1.90×10^8 ;	lg *N* =	8.28
10^{-7}	*24*	*25*	$N_o = N / 10$;	lg N_o =	7.28
			$7.17 \le \lg N_o \le 7.70$?	☒ yes	☐ no

Test:

Conc. of the product	Contact time	*Vc*1	*Vc*2	*Na* ($\overline{n} \times 10$)	lg *Na*	lg *R* (lg N_o - lg *Na*)
1:50	*5 min*	*0*	*0*	<140	<2.15	>5.13

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

Registered address: 128 City Road,
London, EC1V 2NX, United Kingdom

A company registered in England and Wales
Company number: 10031406

Results: EN 1656:2019

RST 003 (Issue 6)

Test organism:	*Enterococcus hirae*		(DSM 3320)
Date of test:	12 November 2024	Test temperature:	10°C ± 1°C
Interfering substance:	10.0 g/l bovine albumin + 10.0 g/l yeast extract		
Dilution-neutralisation method:	Pour plate	Number of plates:	1 / ml
Neutraliser:	B	Incubation temperature:	36°C ± 1°C

Validation and controls:

Validation suspension (Nv_o)			Experimental conditions control (*A*)			Neutraliser or filtration control (*B*)			Method validation (*C*) Product conc.: *1:50*		
*Vc*1	*34*	$\overline{n}$ =	*Vc*1	*41*	$\overline{n}$ =	*Vc*1	*40*	$\overline{n}$ =	*Vc*1	*30*	$\overline{n}$ =
*Vc*2	*40*	37	*Vc*2	*43*	42	*Vc*2	*35*	37.5	*Vc*2	*31*	30.5
$30 \le \overline{n}$ of $Nv_o \le 160$?			$\overline{n}$ of $A \ge 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $B \ge 0.5 \times \overline{n}$ of Nv_o ?			$\overline{n}$ of $C \ge 0.5 \times \overline{n}$ of Nv_o ?		
☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no			☒ yes ☐ no		

Test suspension (*N* and N_o):

N	*Vc*1	*Vc*2			
10^{-6}	*156*	*204*	$\overline{n}_{wm}$ = 1.82×10^8 ;	lg *N* =	8.26
10^{-7}	*23*	*13*	$N_o = N / 10$;	lg N_o =	7.26
			$7.17 \le \lg N_o \le 7.70$?	☒ yes	☐ no

Test:

Conc. of the product	Contact time	*Vc*1	*Vc*2	*Na* ($\overline{n} \times 10$)	lg *Na*	lg *R* (lg N_o - lg *Na*)
1:50	*5 min*	*0*	*0*	<140	<2.15	>5.11

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

Registered address: 128 City Road,
London, EC1V 2NX, United Kingdom

A company registered in England and Wales
Company number: 10031406

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
B	number of survivors per ml in the neutraliser or filtration control mixture
C	number of survivors per ml in the method validation mixture

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