

**Report:** BIS.18M044.VB

**Issued:** 1 February 2019

**Page:** 1 of 8

**Test Report:**

**EN 1656:2009**

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, United Kingdom

**Identification of the client:**

Biosan Ltd  
PMJ House, Highlands Road, Shirley, Solihull, B90 4ND,  
United Kingdom

**Identification of the sample:**

18M/044

Name of the product:

Anolyte Lab Test Sample

Batch number/reference and  
expiry date (if available):

Lot no: B/RW/001, Sample date: 14/12/2018

Date of delivery:

19 December 2018

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.

**Report:** BIS.18M044.VB

**Issued:** 1 February 2019

**Page:** 2 of 8

**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)  
Neutraliser validation: Validated in accordance with EN 1656:2009 (5.5.2)

**Experimental conditions:**

Period of analysis: 29 January 2019 to 31 January 2019  
Product test concentration(s): Neat, 1:5  
Diluent used for product test solution(s): Hard water (N/A for neat)  
Contact time(s) 30 min  $\pm$  10 s  
Test temperature(s) 10°C  $\pm$  1°C  
Interfering substance 3.0 g/l bovine albumin (low-level soiling)  
Temperature of incubation 36°C  $\pm$  1°C  
Identification of the bacterial strain(s) used  
*Pseudomonas aeruginosa* (DSM 939)  
*Proteus vulgaris* (DSM 30118)  
*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 1656:2009 (5.4.2) or EN 1656:2009 (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 product shall demonstrate at least a 5 decimal log (lg) reduction against all test organisms.

**Conclusion:**

According to EN 1656:2009, Anolyte Lab Test Sample possesses bactericidal activity when tested neat, and at a concentration of 1:5, with a contact time of 30 minutes at 10°C under high-level soiling against all of the referenced strains of *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus* and *Enterococcus hirae*.

**Report prepared by:**

Signed:



Name:

Tony Watson

Position:

General Manager

Date:

1 February 2019

**Approved by:**

Signed:



Name:

Gareth Bayliss

Position:

Laboratory Manager

Date:

1 February 2019

**Results:** EN 1656:2009

Test organism: *Pseudomonas aeruginosa* (DSM 939)  
 Date of test: 29 January 2019  
 Test temperature: 10°C ± 1°C Incubation temperature: 36°C ± 1°C  
 Dilution-neutralisation method: Pour plate Number of plates: 1 / ml  
 Neutraliser: A Test conditions: Low-level soiling

**Validation and controls:**

Validation suspension ( $N_{v0}$ )			Experimental conditions control (A)			Neutraliser or filtration control (B)			Method validation (C) Product conc.: <i>Neat</i>		
Vc1	55	$\bar{x} =$	Vc1	57	$\bar{x} =$	Vc1	60	$\bar{x} =$	Vc1	64	$\bar{x} =$
Vc2	61	58	Vc2	63	60	Vc2	60	60	Vc2	61	62.5
30 ≤ $\bar{x}$ of $N_{v0}$ ≤ 160 ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			$\bar{x}$ of A ≥ 0.5 × $\bar{x}$ of $N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			$\bar{x}$ of B ≥ 0.5 × $\bar{x}$ of $N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			$\bar{x}$ of C ≥ 0.5 × $\bar{x}$ of $N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no		

**Test suspension ( $N$  and  $N_0$ ):**

$N$	Vc1	Vc2	$\bar{x}$ wm = 3.70 × 10 <sup>8</sup> ; $N_0 = N / 10$ ; 7.17 ≤ lg $N_0$ ≤ 7.70 ?
10 <sup>-6</sup>	>330	>330	lg $N$ = 8.57 lg $N_0$ = 7.57
10 <sup>-7</sup>	38	36	<input checked="" type="checkbox"/> yes <input type="checkbox"/> no

**Test:**

Conc. of the product	Contact time	Vc1	Vc2	$N_a$ ( $\bar{x} \times 10$ )	lg $N_a$	lg $R$ (lg $N_0$ - lg $N_a$ )
<i>Neat</i>	30 min	0	0	<140	<2.15	>5.42
1:5	30 min	0	0	<140	<2.15	>5.42

**Results:** EN 1656:2009

Test organism:	<i>Proteus vulgaris</i>	(DSM 30118)
Date of test:	29 January 2019	
Test temperature:	10°C ± 1°C	Incubation temperature: 36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates: 1 / ml
Neutraliser:	A	Test conditions: Low-level soiling

**Validation and controls:**

Validation suspension ( $N_{V_0}$ )			Experimental conditions control (A)			Neutraliser or filtration control (B)			Method validation (C) Product conc.: <i>Neat</i>		
Vc1	69	$\bar{x} =$	Vc1	67	$\bar{x} =$	Vc1	71	$\bar{x} =$	Vc1	69	$\bar{x} =$
Vc2	74	71.5	Vc2	69	68	Vc2	71	71	Vc2	73	71
30 ≤ $\bar{x}$ of $N_{V_0}$ ≤ 160 ?			$\bar{x}$ of A ≥ 0.5 × $\bar{x}$ of $N_{V_0}$ ?			$\bar{x}$ of B ≥ 0.5 × $\bar{x}$ of $N_{V_0}$ ?			$\bar{x}$ of C ≥ 0.5 × $\bar{x}$ of $N_{V_0}$ ?		
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no		

**Test suspension ( $N$  and  $N_0$ ):**

$N$	Vc1	Vc2	$\bar{x}$ $w_m = 4.05 \times 10^8$ ; $\lg N = 8.61$ $N_0 = N / 10$ ; $\lg N_0 = 7.61$ 7.17 ≤ $\lg N_0$ ≤ 7.70 ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no
10 <sup>-6</sup>	>330	>330	
10 <sup>-7</sup>	39	42	

**Test:**

Conc. of the product	Contact time	Vc1	Vc2	$N_a$ ( $\bar{x} \times 10$ )	$\lg N_a$	$\lg R$ ( $\lg N_0 - \lg N_a$ )
<i>Neat</i>	30 min	0	0	<140	<2.15	>5.46
1:5	30 min	0	0	<140	<2.15	>5.46

**Results:** EN 1656:2009

Test organism:	<i>Staphylococcus aureus</i>	(DSM 799)
Date of test:	29 January 2019	
Test temperature:	10°C ± 1°C	Incubation temperature: 36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates: 1 / ml
Neutraliser:	A	Test conditions: Low-level soiling

**Validation and controls:**

Validation suspension ( $N_{v0}$ )			Experimental conditions control (A)			Neutraliser or filtration control (B)			Method validation (C) Product conc.: <i>Neat</i>		
Vc1	72	$\bar{x}$ =	Vc1	70	$\bar{x}$ =	Vc1	73	$\bar{x}$ =	Vc1	70	$\bar{x}$ =
Vc2	69	70.5	Vc2	67	68.5	Vc2	72	72.5	Vc2	70	70
30 ≤ $\bar{x}$ of $N_{v0}$ ≤ 160 ?			$\bar{x}$ of A ≥ 0.5 × $\bar{x}$ of $N_{v0}$ ?			$\bar{x}$ of B ≥ 0.5 × $\bar{x}$ of $N_{v0}$ ?			$\bar{x}$ of C ≥ 0.5 × $\bar{x}$ of $N_{v0}$ ?		
<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no			<input checked="" type="checkbox"/> yes <input type="checkbox"/> no		

**Test suspension ( $N$  and  $N_0$ ):**

$N$	Vc1	Vc2	$\bar{x}$ wm = $4.65 \times 10^8$ ; $\lg N = 8.67$ $N_0 = N / 10$ ; $\lg N_0 = 7.67$ $7.17 \leq \lg N_0 \leq 7.70$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no
$10^{-6}$	>330	>330	
$10^{-7}$	49	44	

**Test:**

Conc. of the product	Contact time	Vc1	Vc2	$N_a$ ( $\bar{x} \times 10$ )	$\lg N_a$	$\lg R$ ( $\lg N_0 - \lg N_a$ )
<i>Neat</i>	30 min	0	0	<140	<2.15	>5.52
1:5	30 min	0	0	<140	<2.15	>5.52

**Results:** EN 1656:2009

Test organism:	<i>Enterococcus hirae</i>	(DSM 3320)
Date of test:	29 January 2019	
Test temperature:	10°C ± 1°C	Incubation temperature: 36°C ± 1°C
Dilution-neutralisation method:	Pour plate	Number of plates: 1 / ml
Neutraliser:	A	Test conditions: Low-level soiling

**Validation and controls:**

Validation suspension ( $N_{v0}$ )			Experimental conditions control (A)			Neutraliser or filtration control (B)			Method validation (C) Product conc.: <i>Neat</i>		
Vc1	52	$\bar{x} =$ 56	Vc1	63	$\bar{x} =$ 60	Vc1	64	$\bar{x} =$ 62	Vc1	54	$\bar{x} =$ 56.5
Vc2	60		Vc2	57		Vc2	60		Vc2	59	
30 ≤ $\bar{x}$ of $N_{v0}$ ≤ 160 ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			$\bar{x}$ of A ≥ 0.5 × $\bar{x}$ of $N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			$\bar{x}$ of B ≥ 0.5 × $\bar{x}$ of $N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no			$\bar{x}$ of C ≥ 0.5 × $\bar{x}$ of $N_{v0}$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no		

**Test suspension ( $N$  and  $N_0$ ):**

$N$	Vc1	Vc2	$\bar{x}$ wm = $2.69 \times 10^8$ ; $\lg N = 8.43$ $N_0 = N / 10$ ; $\lg N_0 = 7.43$ $7.17 \leq \lg N_0 \leq 7.70$ ? <input checked="" type="checkbox"/> yes <input type="checkbox"/> no
$10^{-6}$	267	272	
$10^{-7}$	27	25	

**Test:**

Conc. of the product	Contact time	Vc1	Vc2	$N_a$ ( $\bar{x} \times 10$ )	$\lg N_a$	$\lg R$ ( $\lg N_0 - \lg N_a$ )
<i>Neat</i>	30 min	0	0	<140	<2.15	>5.28
1:5	30 min	0	0	<140	<2.15	>5.28

**Explanations:**

- $V_c$  count per ml (one plate or more)
- $\bar{x}$  average of  $V_{c1}$  and  $V_{c2}$  (1 + 2 duplicate)
- $\bar{x}_{wm}$  weighted mean of  $\bar{x}$
- $N$  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 ( $N_o = N / 10$ )
- $N_a$  number of survivors per ml in the test mixture at the end of the contact time (before neutralisation or filtration)
- $R$  reduction ( $\lg R = \lg N_o - \lg N_a$ )
- $N_v$  number of cells per ml in the validation suspension
- $N_{v_o}$  number of cells in the validation mixtures at the beginning of the contact time ( $N_{v_o} = N_v / 10$ )
- $A$  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