A quantitative suspension test for the evaluation of the bactericidal activity provided by 20/30 Labs Ltd using SOP L067 [Modified].

Tested according to BS EN1276: 2019

Date: 15/05/2020
Report Reference: 15732
Issue: 1

Prepared by: Joe Fitzsimons, Research Scientist, 20/30 Labs

Test Laboratory:

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Identification of Sample:

Product name: Biosan (4)

Batch number: S1/07/20

Expiry date: Not Given

Date of delivery: 2020/04/30

Client: Biosan Limited

Storage conditions: Room Temperature

Appearance of product: Clear Solution

Active substance(s) & their concentrations: Hypochlorous acid (HOCl), 0.04-0.05%

Product diluent recommended by the manufacturer for use: N/A – Product Ready to use.

Experimental Conditions:

Period of analysis: 12/05/2020 – 15/05/2020

Method justification: Dilution Neutralisation – Satisfactory recovery on all neutralisation controls

Product diluent: Water

Product test concentrations: 80%, 50%, 1%

Appearance of product dilutions: Clear Solutions

Contact time: 10 seconds

Test temperature: 20°C

Interfering substances: 0.3g/L Bovine Albumin = Clean Conditions

Stability and appearance of test mixtures: Clear and homogenous

Incubation temperature: 36°C

Neutraliser: 10g/L Sodium Thiosulphate, 30g/L Polysorbate 80, 3g/L Lecithin, in Water

Test-organisms: *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus hirae, Escherichia coli*

Results – Test Organism: *Pseudomonas aeruginosa* ATCC 15442

Date of test: 12/05/2020

Method: Standard Dilution-Neutralisation, Spread Plate

Neutraliser: 10g/L Sodium Thiosulphate, 30g/L Polysorbate 80, 3g/L Lecithin, in Water

Incubation temperature: 36°C

Product Diluent: Water

Media Used / Batch No. : TSA / 4255485

Responsible person: Joseph Fitzsimons

Test temperature: 20°C

Interfering substance: Clean Conditions - 0.3g/L Bovine Albumin

Appearance of test mixtures: Clear and homogenous

Number of plates: 2 per V_C value

Validation and Control Enumerations

Validation Suspension (N_{V0})		$\bar{x}$ =
V_{C1}	84	80.5
	43 + 41	
V_{C2}	77	
	38 + 39	
30 ≤ $\bar{x}$ of N_{V0} ≤ 160 ?		
PASS		

Experimental Conditions (***A***)		$\bar{x}$ =	Neutraliser Control (***B***)		$\bar{x}$ =	Method Validation (***C***)		$\bar{x}$ =
V_{C1}	59	52.5	V_{C1}	44	46	V_{C1}	50	56.5
	33 + 26			20 + 24			26 + 24	
V_{C2}	46		V_{C2}	48		V_{C2}	63	
	16 + 30			27 + 21			31 + 32	
$\bar{x}$ of ***A*** is ≥ 0.5 $\bar{x}$ of N_{V0} ?			$\bar{x}$ of ***B*** is ≥ 0.5 $\bar{x}$ of N_{V0} ?			$\bar{x}$ of ***C*** is ≥ 0.5 $\bar{x}$ of N_{V0} ?		
PASS			**PASS**			**PASS**		

Test Suspension and Test Results

Test Suspension (***N***) 10^{-6}:		Test Suspension (***N***) 10^{-7}:		$\bar{x}_{wm}$ of ***N*** =	$3.5x10^8$
				N₀ = ***N***/10 =	$3.5x10^7$
V_{C1}	339	V_{C1}	43	lg ***N*** =	8.54
	166 + 173		24 + 19	lg ***N₀*** =	7.54
V_{C2}	353	V_{C2}	38	$7.17 \leq \lg N_0 \leq 7.70$?	**PASS**
	163 + 190		21 + 17		

Real Conc. Of Product (%)*	Contact time (seconds)	Dilution step	V_{C1}		V_{C2}		***N*ₐ** = ($\bar{x}$ or $\bar{x}_{wm}$) x 10	lg ***N*ₐ**	lg ***R***
80	10	Neat	0 + 0	0	0 + 0	0	$<1.4x10^2$	<2.15	>5.40
		10^{-1}	0 + 0	0	0 + 0	0			
50	10	Neat	0 + 0	0	0 + 0	0	$<1.4x10^2$	<2.15	>5.40
		10^{-1}	0 + 0	0	0 + 0	0			
1	10	Neat	>330 + >330	>660	>330 + >330	>660	$>6.6x10^4$	>4.82	<2.72
		10^{-1}	>330 + >330	>660	>330 + >330	>660			

*Active to Non-Active ranges

***V*꜀** = Count per 1ml (one plate or more)

$\bar{\mathbf{X}}$ = Mean of V_{C1} and V_{C2}

$\bar{\mathbf{X}}_{\mathbf{wm}}$ = Weighted mean of $\bar{\mathbf{X}}$

R = Reduction ($\lg R = \lg N_0 - \lg N_a$)

Special Remarks: N/A

Results – Test Organism: *Staphylococcus aureus* ATCC 6538

Date of test: 12/05/2020

Method: Standard Dilution-Neutralisation, Spread Plate

Neutraliser: 10g/L Sodium Thiosulphate, 30g/L Polysorbate 80, 3g/L Lecithin, in Water

Incubation temperature: 36°C

Product Diluent: Water

Media Used / Batch No. : TSA / 4255485

Responsible person: Joseph Fitzsimons

Test temperature: 20°C

Interfering substance: Clean Conditions - 0.3g/L Bovine Albumin

Appearance of test mixtures: Clear and homogenous

Number of plates: 2 per V_C value

Validation and Control Enumerations

Validation Suspension (***N_{V0}***)		$\bar{x}$ =
V_{C1}	67	74
	37 + 30	
V_{C2}	81	
	36 + 45	
30 ≤ $\bar{x}$ of ***N_{V0}*** ≤ 160 ?		
PASS		

Experimental Conditions (***A***)		$\bar{x}$ =	Neutraliser Control (***B***)		$\bar{x}$ =	Method Validation (***C***)		$\bar{x}$ =
V_{C1}	88	76.5	V_{C1}	68	70	V_{C1}	69	76.5
	52 + 36			29 + 39			31 + 38	
V_{C2}	65		V_{C2}	72		V_{C2}	84	
	32 + 33			33 + 39			44 + 40	
$\bar{x}$ of ***A*** is ≥ 0.5 $\bar{x}$ of ***N_{V0}*** ?			$\bar{x}$ of ***B*** is ≥ 0.5 $\bar{x}$ of ***N_{V0}*** ?			$\bar{x}$ of ***C*** is ≥ 0.5 $\bar{x}$ of ***N_{V0}*** ?		
PASS			**PASS**			**PASS**		

Test Suspension and Test Results

Test Suspension (***N***) 10^{-6}:		Test Suspension (***N***) 10^{-7}:		$\bar{x}_{wm}$ of ***N*** =	$3.2x10^8$
				N₀ = ***N***/10 =	$3.2x10^7$
V_{C1}	336	V_{C1}	32	lg ***N*** =	8.51
	158 + 178		13 + 19	lg ***N₀*** =	7.51
V_{C2}	316	V_{C2}	30	7.17 ≤ lg ***N₀*** ≤ 7.70 ?	**PASS**
	138 + 178		14 + 16		

Real Conc. Of Product (%)*	Contact time (seconds)	Dilution step	V_{C1}		V_{C2}		***N*ₐ** = ($\bar{x}$ or $\bar{x}_{wm}$) x 10	lg ***N*ₐ**	lg ***R***
80	10	Neat	0 + 0	0	0 + 0	0	$<1.4x10^2$	<2.15	>5.36
		10^{-1}	0 + 0	0	0 + 0	0			
50	10	Neat	0 + 0	0	0 + 0	0	$<1.4x10^2$	<2.15	>5.36
		10^{-1}	0 + 0	0	0 + 0	0			
1	10	Neat	>330 + >330	>660	>330 + >330	>660	$>6.6x10^4$	>4.82	<2.69
		10^{-1}	>330 + >330	>660	>330 + >330	>660			

*Active to Non-Active ranges

***V*c** = Count per 1ml (one plate or more)

$\bar{\mathbf{X}}$ = Mean of V_{C1} and V_{C2}

$\bar{\mathbf{X}}_{\mathbf{wm}}$ = Weighted mean of $\bar{\mathbf{X}}$

R = Reduction (lg R = lgN_0 – lgN_a)

Special Remarks: N/A

Results – Test Organism: *Enterococcus hirae* ATCC 10541

Date of test: 13/05/2020

Method: Standard Dilution-Neutralisation, Spread Plate

Neutraliser: 10g/L Sodium Thiosulphate, 30g/L Polysorbate 80, 3g/L Lecithin, in Water

Incubation temperature: 36°C

Product Diluent: Water

Media Used / Batch No. : TSA / 4255485

Responsible person: Joseph Fitzsimons

Test temperature: 20°C

Interfering substance: Clean Conditions - 0.3g/L Bovine Albumin

Appearance of test mixtures: Clear and homogenous

Number of plates: 2 per V_C value

Validation and Control Enumerations

Validation Suspension (N_{V0})		x̄ =
V_{C1}	70	68
	45 + 25	
V_{C2}	66	
	34 + 32	
30 ≤ $\bar{x}$ of N_{V0} ≤ 160 ?		
PASS		

Experimental Conditions (*A*)		x̄ =	Neutraliser Control (*B*)		x̄ =	Method Validation (*C*)		x̄ =
V_{C1}	78	77	V_{C1}	82	80	V_{C1}	70	75
	31 + 47			36 + 46			31 + 39	
V_{C2}	76		V_{C2}	78		V_{C2}	80	
	41 + 35			43 + 35			37 + 43	
x̄ of ***A*** is ≥ 0.5 $\bar{x}$ of N_{V0} ?			x̄ of ***B*** is ≥ 0.5 $\bar{x}$ of N_{V0} ?			x̄ of ***C*** is ≥ 0.5 $\bar{x}$ of N_{V0} ?		
PASS			**PASS**			**PASS**		

Test Suspension and Test Results

Test Suspension (***N***) 10^{-6}:		Test Suspension (***N***) 10^{-7}:		$\bar{x}_{wm}$ of ***N*** =	$2.9x10^8$
				N₀ = ***N***/10 =	$2.9x10^7$
V_{C1}	301	V_{C1}	24	lg ***N*** =	8.46
	132 + 169		13 + 11	lg ***N₀*** =	7.46
V_{C2}	282	V_{C2}	26	7.17 ≤ lg ***N₀*** ≤ 7.70 ?	**PASS**
	158 + 124		15 + 11		

Real Conc. Of Product (%)*	Contact time (seconds)	Dilution step	V_{C1}		V_{C2}		***N*ₐ** = ($\bar{x}$ or $\bar{x}_{wm}$) x 10	lg ***N*ₐ**	lg ***R***
80	10	Neat	0 + 0	0	0 + 0	0	$<1.4x10^2$	<2.15	>5.32
		10^{-1}	0 + 0	0	0 + 0	0			
50	10	Neat	0 + 0	0	0 + 0	0	$<1.4x10^2$	<2.15	>5.32
		10^{-1}	0 + 0	0	0 + 0	0			
1	10	Neat	>330 + >330	>660	>330 + >330	>660	$>6.6x10^4$	>4.82	<2.64
		10^{-1}	>330 + >330	>660	>330 + >330	>660			

*Active to Non-Active ranges

***V*c** = Count per 1ml (one plate or more)

$\bar{\mathbf{X}}$ = Mean of V_{C1} and V_{C2}

$\bar{\mathbf{X}}_{\mathbf{wm}}$ = Weighted mean of $\bar{\mathbf{X}}$

R = Reduction (lg R = lgN_0 – lgN_a)

Special Remarks: N/A

Results – Test Organism: *Escherichia coli* NTCC 10538

Date of test: 12/05/2020

Method: Standard Dilution-Neutralisation, Spread Plate

Neutraliser: 10g/L Sodium Thiosulphate, 30g/L Polysorbate 80, 3g/L Lecithin, in Water

Incubation temperature: 36°C

Product Diluent: Water

Media Used / Batch No. : TSA / 4255485

Responsible person: Joseph Fitzsimons

Test temperature: 20°C

Interfering substance: Clean Conditions - 0.3g/L Bovine Albumin

Appearance of test mixtures: Clear and homogenous

Number of plates: 2 per V_C value

Validation and Control Enumerations

Validation Suspension (N_{V0})		x̄ =
V_{C1}	72	68
	34 + 38	
V_{C2}	64	
	27 + 37	
30 ≤ $\bar{x}$ of N_{V0} ≤ 160 ?		
PASS		

Experimental Conditions (*A*)		x̄ =	Neutraliser Control (*B*)		x̄ =	Method Validation (*C*)		x̄ =
V_{C1}	77	73.5	V_{C1}	60	60	V_{C1}	62	59.5
	38 + 39			36 + 24			36 + 26	
V_{C2}	70		V_{C2}	60		V_{C2}	57	
	32 + 38			32 + 28			26 + 31	
x̄ of ***A*** is ≥ 0.5 $\bar{x}$ of N_{V0} ?			x̄ of ***B*** is ≥ 0.5 $\bar{x}$ of N_{V0} ?			x̄ of ***C*** is ≥ 0.5 $\bar{x}$ of N_{V0} ?		
PASS			**PASS**			**PASS**		

Test Suspension and Test Results

Test Suspension (***N***) 10^{-6}:		Test Suspension (***N***) 10^{-7}:		$\bar{x}_{wm}$ of ***N*** =	$2.5x10^8$
				N₀ = ***N***/10 =	$2.5x10^7$
V_{C1}	250	V_{C1}	30	lg ***N*** =	8.40
	124 + 126		12 + 18	lg ***N₀*** =	8.40
V_{C2}	252	V_{C2}	17	7.17 ≤ lg ***N₀*** ≤ 7.70 ?	**PASS**
	136 + 116		9 + 8		

Real Conc. Of Product (%)*	Contact time (seconds)	Dilution step	V_{C1}		V_{C2}		***N*ₐ** = ($\bar{x}$ or $\bar{x}_{wm}$) x 10	lg ***N*ₐ**	lg ***R***
80	10	Neat	0 + 0	0	0 + 0	0	$<1.4x10^2$	<2.15	>5.25
		10^{-1}	0 + 0	0	0 + 0	0			
50	10	Neat	0 + 0	0	0 + 0	0	$<1.4x10^2$	<2.15	>5.25
		10^{-1}	0 + 0	0	0 + 0	0			
1	10	Neat	>330 + >330	>660	>330 + >330	>660	$>6.6x10^4$	>4.82	<2.58
		10^{-1}	>330 + >330	>660	>330 + >330	>660			

*Active to Non-Active ranges

***V*c** = Count per 1ml (one plate or more)

$\bar{\mathbf{X}}$ = Mean of V_{C1} and V_{C2}

$\bar{\mathbf{X}}_{\mathbf{wm}}$ = Weighted mean of $\bar{\mathbf{X}}$

R = Reduction (lg R = lgN_0 – lgN_a)

Special Remarks: N/A

Conclusion

For the product Biosan (4) (batch S1/07/20), the bactericidal concentration determined according to EN1276, under clean conditions at 20°C using *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus hirae,* and *Escherichia coli*, is 50%, when using a 10 second contact time.

Method deviation: A contact time of 10 seconds is below the specified minimum contact time for hand sanitisers in EN1276

Tested by / Authorised by:

Tested by:	Authorised by:
Name: Joseph Fitzsimons	Name: James Clarke
Title: Research Scientist	Title: Head of Innovation